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GIANT CELL PNEUMONIA WITH INCLUSIONS

A LESION COMMON TO HECHT'S DISEASE, DISTEMPER AND MEASLES *

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Giant cell pneumonia was first described by Hecht [1] in 1910. Clinically, there is little to distinguish the condition from other types of subacute or chronic pneumonia in infants. Measles, tuberculosis, or syphilis may be associated with it, but it often occurs in an apparently uncomplicated form. Although Hecht found the condition in 27 instances among 162 cases of pneumonia in infants and young children, only a few cases have been reported since 1910.

The diagnosis of giant cell pneumonia is made on the basis of the histological picture. The lesion is an interstitial pneumonitis, the most important diagnostic feature of which is the formation of large multinucleated cells by proliferation and fusion of the cells which line the alveoli, alveolar ducts, and bronchioles. As has been pointed out by Moore and Gross,[2] giant cells of several different types may occur in the lungs of infants under a variety of conditions. Many observers, however, including Chown,[3] have felt that the picture of Hecht's giant cell pneumonia,[1] in its fully developed form, represents a distinct pathological entity. Chown believed that the cause of the condition might be vitamin A deficiency. This etiological theory was soundly based on histological evidence, and will be discussed later.

In examining the sections of a typical case of giant cell pneumonia (previously unreported) numerous and prominent cytoplasmic inclusions were noted in giant cells, and also in alveolar and bronchial lining cells. This led us to examine sections of 4 other cases of giant cell pneumonia. Three of these had been reported previously (Chown,[3] Masson and Paré,[4] Karsner and Meyers [5]) while the fourth (unpublished) was made available by Dr. Masson. In all 4 of these cases, many cytoplasmic inclusions, similar to those seen in the first case, were present, and intranuclear inclusions were also found in large numbers.

* Received for publication, January 31, 1944.

The striking morphological resemblance of these cytoplasmic and nuclear inclusions to those associated with distemper in animals led us to make detailed comparative studies of the two conditions. The occurrence of giant cells in lymphoid tissue in prodromal measles suggested the inclusion of tissue from fatal measles in our study.

Observations in Giant Cell Pneumonia

Case 1

(Previously unreported.) B. C., a Negro female infant, was born spontaneously at the Homer G. Phillips Hospital after 33 weeks of gestation. Crying was spontaneous; respiration and color were good. The birth weight was 2,370 gm. The mother was described as well nourished and in good health. She had attended a prenatal clinic and was on a supposedly adequate diet. She had one child living and well, 18 months of age. The infant was placed on the breast after the first 8 hours and fed six times daily for the next 3 days. Diarrhea developed on the 4th day. The infant was placed on a formula of condensed milk and the diarrhea ceased, but the weight loss continued. On the 9th day the infant became markedly cyanotic with rapid respirations. Examination at this time failed to reveal any pulmonary findings. X-ray examination of the chest was negative; Kahn test of the blood was negative; hemoglobin, 90 per cent; erythrocytes, 5,200,000; white blood cells, 7,400; temperature, 98.8° F. (rectal). Medication consisted of 25 cc. of Hartman's solution, subcutaneously, and 15 cc. of whole blood. Following this, clinical improvement was noted. Respirations became slower and less labored. The color became normal; temperature remained normal. On the 14th hospital day the infant again became cyanotic and dyspneic, and expired after 3½ hours.

Necropsy was performed 6 hours post-mortem. Subcutaneous and perivisceral fat was scanty in amount. There was evidence of dehydration. Exclusive of the lungs, the findings were as follows: Heart, spleen, stomach and intestines, adrenals, thymus, brain, normal; liver and kidneys, cloudy swelling. The lungs grossly showed glistening, moist pleural surfaces. They were dark red at the bases, tending to fade to a bright red at the apices. They were somewhat collapsed, rather firm in consistency and cut with increased resistance. The cut surface was moist and of a deeply congested homogeneous appearance. The grayish nodules characteristic of consolidation were noticeably absent. The bronchi contained a thick, reddish gray, viscid material.

Microscopical examination of the lungs (hematoxylin and eosin stain) showed intense congestion of the alveolar capillaries, and infiltration of the alveolar walls by many mononuclear cells, together with rare neutrophils. Many alveoli were collapsed, but those which were patent contained a serofibrinous exudate in which a few large, vacuolated mononuclear cells were present. The bronchioles contained a fibrinous or mucoid exudate, with desquamated epithelial cells and occasionally with moderate numbers of neutrophils.

On low-power examination, 10 to 15 large multinucleated cells were present in nearly every field (Fig. 1). On high-power study, these

giant cells were seen to be derived from the lining cells of the alveoli, alveolar ducts and bronchioles. The alveolar lining cells, when not forming giant cells, commonly showed cuboidal metaplasia and were often in mitosis.

Cytoplasmic inclusions were present in about one-half of the giant cells, in the epithelium of most of the bronchioles, and in many of the hypertrophied alveolar lining cells. A giant cell showing 15 to 20 nuclei often showed as many as 8 to 10 inclusions, and occasionally there were more inclusions than nuclei. The inclusions varied from light pink to reddish purple, depending on the relative intensity of the staining with hematoxylin and eosin. In the bronchioles, they were most often found in those areas where the epithelium projected into the lumen in the form of a multinucleated syncytial bud (Fig. 2). In the bronchial epithelium, the inclusions ranged from 2 to 10 μ in greatest dimension, and were most often roughly spherical or ovoid. In the giant cells, they ranged up to 25 μ in greatest dimension, and showed greater morphological variation (Fig. 3). Frequently small vacuoles could be seen in the substance of the inclusions, and many of the medium-sized spherical forms resembled Negri bodies rather closely. The inclusions were frequently paranuclear in location, and occasionally the adjacent nuclei were indented.

Prolonged study showed no definite nuclear inclusions. No inclusions of any type were found in sections of liver, pancreas, or kidney (pelvic epithelium not included). A few cytoplasmic inclusions of moderate size were found in reticulo-endothelial cells in the spleen.

Case 2

(Previously reported by Masson and Paré.[4]) A 9-month-old infant was admitted to the hospital with a cough of 1 month's duration, and dyspnea. A rash occurred at the beginning of the illness. The clinical diagnosis was pulmonary tuberculosis and tuberculous meningitis. Death occurred about 4 weeks after admission to the hospital.

Autopsy showed miliary tuberculosis, rickets, caseous tuberculous lymphadenitis, and bronchopneumonia with bronchiectasis.

Microscopical examination of the lungs stained with hematoxylin and eosin and by the Giemsa method showed many small tubercles with caseous centers and giant cells of the type commonly seen in tuberculosis. In addition to this picture, however, syncytial multinucleated cells arising from alveolar and bronchiolar lining cells were numerous, and in spite of the presence of the lesions of tuberculosis, the diagnosis of Hecht's giant cell pneumonia [1] could be made with confidence. The giant cells of the "tuberculous" type could be distinguished, in most instances, from those characteristic of giant cell

pneumonia by the tendency of the latter to be attached to alveolar and bronchiolar walls (or, if lying free, by the presence of elongated strands of cytoplasm) and by the commonly central location of their nuclei. In many of the giant cells of the latter type, cytoplasmic inclusions identical with those described in case 1 were present in large numbers.

Nuclear inclusions were present also in large numbers in the nuclei of giant cells, alveolar lining cells and bronchiolar epithelial cells (Fig. 4). These nuclear inclusions most commonly filled only one-fourth to one-third of the nucleus. They were homogeneous, eosinophilic, and rounded or oval. The nuclear inclusions were often stained less intensely than the cytoplasmic, as though the nuclear membrane had acted as a barrier to the penetration of the stain. By overstaining with eosin, the nuclear inclusions could often be brought out more clearly, at the expense of definition of the cytoplasmic bodies. In cells where nucleoli were present, they were readily differentiated from the latter structures by differences in size and staining characteristics. The inclusions were nearly always surrounded by a wide zone of clearing in the nuclear chromatin. Many of the affected nuclei were enlarged, and occasionally a suggestion of margination of the nuclear chromatin was seen. The latter feature, however, was inconspicuous.

Case 3

(Previously unpublished. Made available through the courtesy of Dr. Masson.) The patient was a girl, 2½ years old, who had had subacute bronchopneumonia of about 6 weeks' duration, with suppurative pneumococcal pleuritis.

Microscopical examination of the lungs showed interstitial pneumonitis. In many areas the alveolar walls were crowded with mononuclear cells, while the alveoli contained serous fluid with only a few mononuclear cells. In other areas, alveoli and bronchioles contained a purulent exudate, probably the result of secondary infection. There was a striking tendency for the alveolar lining cells to undergo cuboidal metaplasia. Giant cells of the type described in cases 1 and 2 were present, but in smaller numbers than in the other 4 cases included in this series. In several areas, only one or two giant cells were present in ten low-power fields. The giant cells showed a tendency to be most numerous in the regions adjacent to interlobular septa.

In alveolar lining cells, bronchial epithelium, and giant cells (Fig. 5), cytoplasmic and nuclear inclusions apparently identical with those described in cases 1 and 2 were present in large numbers. They were eosinophilic when stained with either hematoxylin and eosin or Giemsa's stain.

Case 4

(Previously reported by Chown.[8]) The patient was an infant with a previous history of congenital syphilis, which was apparently cured by treatment. At the age of 16 months, spreading bronchopneumonia developed, and this condition terminated fatally 26 days later.

At autopsy the main gross finding was bilateral bronchopneumonia. Microscopical examination of the lungs showed giant cells fully as numerous as in case 1. Histologically, the inflammatory cell reaction was largely mononuclear in some areas, while in other areas many neutrophils were present. It should be pointed out that in certain blocks of lung tissue, giant cells were present only in very small numbers. Nuclear and cytoplasmic inclusions identical with those described in cases 1, 2 and 3 were present in alveolar lining cells, bronchial epithelium and giant cells. Nuclear inclusions were found less uniformly throughout the sections than in cases 2 and 3, but were numerous in localized areas. Cytoplasmic inclusions were more numerous in this case than in any of the others.

Case 5

(Previously reported by Karsner and Meyers.[5]) An 18-month-old infant was admitted to the hospital with severe rickets and diffuse bronchopneumonia. An illness which was probably bronchopneumonia had occurred 1 month before entry, but the patient apparently had recovered. Death occurred after an illness of 2 weeks.

Autopsy showed patchy bronchopneumonia. Cultures from the lung tissue showed staphylococci.

Microscopical examination of the lungs showed numerous giant cells of the type described in the other cases. In most areas, the originally mononuclear reaction was obscured by a reaction of the purulent type. The giant cells, bronchiolar epithelium and alveolar lining cells contained large numbers of cytoplasmic inclusions like those described in the other cases. Nuclear inclusions were present in moderate numbers. It is of interest to note that in a section stained with eosin and methylene blue the inclusions were brightly eosinophilic and prominent, while in a section stained by hematoxylin and eosin the inclusions were made out with difficulty. (Neither of these sections had been restained since 1911.)

No inclusions were found in sections of lymph node, spleen, thymus, or kidney (pelvic mucosa not included). In the single section of liver available for study, five fairly characteristic cytoplasmic inclusions were found in bile duct epithelium. The section was somewhat faded, and it was not possible to be certain that these inclusions were authentic. The question of the presence of inclusions in organs other than the lung in giant cell pneumonia requires further study.

Observations in Distemper

For the histological study of distemper, sections from 63 minks, 22 ferrets and 12 foxes were available. These sections were prepared in 1938, in the course of a study of epidemics of distemper in minks and foxes. The histological picture of distemper virus pneumonia as seen in this material was reported briefly by one of us in 1940.[6] In that report multinucleated cells containing the characteristic inclusions of distemper were described in the lungs, but the possible relationship of the lesion to giant cell pneumonia was not considered at that time. In the present paper, this pulmonary lesion will be described in greater detail.

In minks, the lungs were believed to show the picture of uncomplicated virus infection. The lungs from a few of the minks showed only intense congestion, with cytoplasmic inclusions in the bronchiolar epithelium. These were probably cases in which early death occurred from overwhelming infection. In the great majority of instances, however, patchy areas of consolidation, 1 to 5 mm. in diameter, were found, and in several instances there was almost uniform consolidation of one or more lobes. On microscopical examination, the alveolar walls in the consolidated areas were greatly thickened, and contained large numbers of macrophages and plasma cells. The alveolar lining cells were hypertrophied, and often in mitosis. Large multinucleated cells, obviously derived from the alveolar lining cells by proliferation and fusion, were found in variable numbers in different cases. The alveoli often contained a few mononuclear cells some of which were vacuolated and appeared to be macrophages, while others appeared to be desquamated alveolar lining cells. Neutrophils were inconspicuous. Giant cells often appeared to lie free in the alveoli, but careful study of several sections mounted on the same slide suggested that many of these were still attached to the alveolar walls, and were in reality parts of an extensive irregular syncytium. The bronchiolar epithelium also showed marked metaplasia, with occasional giant cell formation.

In many sections of the lungs of minks, giant cells were prominent in only one or two small areas, particularly in a subpleural position, or adjacent to connective tissue septa. In several, however, giant cells were numerous throughout the slide. In these cases, the resemblance to Hecht's giant cell pneumonia[1] appeared complete in all details (Fig. 6).

In sections of the lungs of ferrets and foxes, a purulent exudate in the alveoli was the rule. This is believed to be the result of secondary bacterial infection.

Metaplasia and proliferation of bronchial and alveolar lining cells

was nearly always obvious, however, and giant cells were present in many cases. In one ferret and in at least one fox, giant cells were sufficiently numerous to suggest "giant cell pneumonia."

Inclusion Bodies

Inclusions characteristic of distemper were found in the epithelium of the bronchioles (Fig. 7) and also in alveolar lining cells and in giant cells derived from cells of these two types (Figs. 8 and 9). In all animals, cytoplasmic inclusions were numerous; nuclear inclusions were more variable, being numerous in the majority of animals and demonstrable in nearly every animal. In 2 minks, however, no nuclear inclusions could be found.

The inclusions in this distemper material were not described in detail in the previous report [6] because it was felt that they had been described adequately by other workers.[7, 8] The most detailed descriptions and the best illustrations of distemper inclusions in the literature probably are to be found in a paper by Green and Evans.[8]

Observations in Fatal Measles

Through the courtesy of Dr. James Denton, sections from 6 fatal cases of measles, which occurred in Panama in 1923 in an epidemic with high mortality, were made available.

Pulmonary Lesions. In 3 of these 6 cases, the lungs showed purulent bronchitis and bronchopneumonia, the appearance of which was in no way suggestive of giant cell pneumonia or of any other type of virus pneumonia. In 2 cases, giant cells with many typical cytoplasmic and nuclear inclusions were present, giving a picture indistinguishable from that of Hecht's giant cell pneumonia or from that of distemper virus pneumonia in animals. In the sixth case, the sections from which were faded, a few giant cells were seen, and a few intracytoplasmic bodies were present which were strongly suggestive of the above described type of inclusion. It is of interest to note that Denton,[8a] in his original description of this material from cases of measles in 1925, stressed the presence of giant cells in the lungs.

Lesions in Other Organs. No giant cells or inclusions were found in sections of heart, spleen, liver, kidney, pancreas, small intestine, or lymph nodes.

Discussion

Inclusions

The distinctive features of the cytological picture associated with distemper infection may be summarized as follows:

1. Occurrence of both nuclear and cytoplasmic inclusions (a rare finding in other virus infections).

2. Presence of inclusions in huge numbers; often 6 or 8 inclusions in a single cell, and 25 or more inclusions in a section of a multinucleated cell.

3. Location of inclusions in alveolar and bronchiolar lining cells, bladder epithelium, and less constantly in bile and pancreatic duct epithelium, skin, and adrenal cortical cells.

4. Cytoplasmic inclusions homogeneous or vacuolated, somewhat resembling Negri bodies; nuclear inclusions rarely if ever vacuolated.

5. Extraordinary range in size of cytoplasmic inclusions (1 to 30 μ) while nuclear inclusions range only from about 1 to 5 μ, rarely filling more than one-third of the nucleus.

6. Marked variation in shape of cytoplasmic inclusions: usually rounded or ovoid, but may be sausage-shaped, vermiform, or irregularly polygonal.

7. Nuclear inclusions more constant in shape, round or ovoid; nearly always occurring singly.

8. Color of inclusions with hematoxylin and eosin stain ranges from light pink to purple, depending on relative intensity of staining with each.

9. Cytoplasmic inclusions often paranuclear, and may cause indentation of adjacent nuclei.

The cytological picture of distemper, when all of its details are considered, is unique and quite unlike that of any other known virus infection. It has generally been regarded as having diagnostic significance.[6,8] If one compares the nuclear and cytoplasmic inclusions seen in the lungs of cases of giant cell pneumonia of infants (Figs. 2, 3, 4 and 5) with those seen in lungs of animals with distemper (Figs. 7, 8 and 9) and with the picture of distemper as illustrated by Green and Evans,[8] and other workers, the apparent cytological identity of the two conditions is evident. On this basis alone, one might be justified in concluding that we are dealing with closely related, if not with identical viruses.

The apparent absence of nuclear inclusions in case 1 is paralleled by the occasional failure to find nuclear inclusions in distemper (observations above and also those by Green and Evans [8]). In the other 4 cases studied, characteristic nuclear as well as cytoplasmic inclusions were present. The characteristic pleomorphism of the cytoplasmic inclusions in case 1, and the histological identity of the pulmonary lesion to that seen in the other 4 cases suggest that all 5 cases are

etiologically identical, and that the absence of demonstrable nuclear inclusions in case 1 is purely fortuitous.

Histological Observations

The essential histological identity of the pulmonary lesions in several cases of pneumonitis of distemper in minks (Fig. 6), foxes, and ferrets with those in giant cell pneumonia (Fig. 1) lends additional support to the view that the two conditions may be closely related etiologically. In the lungs of the animals with distemper, the characteristic giant cells were present in sufficient number to suggest giant cell pneumonia in only a small percentage of the cases. This suggests that the diagnosis of giant cell pneumonia in infants may be missed in many cases because the giant cells are not present in sufficient numbers. It has been pointed out that giant cells were not numerous in case 3, and that in certain blocks of lung tissue in case 4 they were present only in small numbers, while in other blocks they were extremely numerous. In subsequent studies, particular attention should be paid to lungs of infants containing even occasional giant cells, in the absence of a good reason for their presence.

Hypertrophy and cuboidal metaplasia of alveolar lining cells, without giant cell formation, occur in lungs under a wide variety of conditions, including several varieties of virus pneumonia and toxoplasmosis.

Nature of the Virus in Giant Cell Pneumonia

That giant cell pneumonia is a virus disease seems clear beyond a reasonable doubt on the basis of the cytological changes described above. Either the cytoplasmic or the nuclear inclusions alone would strongly support such a conclusion. The occurrence of the two types of inclusions together, often in the same cell, is believed to be conclusive evidence of virus activity.

It has already been pointed out that the inclusions present in the cases of giant cell pneumonia are identical with those of distemper, and that giant cell pneumonia occurs in distemper. Identical nuclear inclusions do not necessarily imply identical or even closely related viruses, and the same may be said of identical cytoplasmic inclusions. When both nuclear and cytoplasmic inclusions are identical, however, and when this identity includes a wide range of morphological variation, it is difficult to believe that the viruses involved are not closely similar.

Strain variations in distemper of such a degree that cross-immunity does not obtain have been described by Slanetz and Smetana.[9] Their

two viruses, although morphologically identical and producing essentially identical clinical pictures in ferrets, were immunologically distinct.

Assuming that the evidence presented here for a close relationship between the virus of giant cell pneumonia and distemper is valid, the exact relationship between the two viruses can be determined only when the former virus is finally isolated in an experimental animal susceptible to distemper.

An important factor in deciding the question of the identity of two viruses—perhaps even more important than the character of the inclusions—is their distribution and their preference for growth in cells of certain types. The cytoplasmic inclusions of canine distemper, for example, may greatly resemble the Negri bodies of rabies. The occurrence of the latter exclusively in the ganglion cells of the brain, however (as well as the absence of intranuclear inclusions), serves to differentiate them sharply.

It has been shown that in the lung the inclusions of giant cell pneumonia are found in cells of the same types as in distemper. Unfortunately, material has not been available for a satisfactory topographical study of inclusions in other organs. Bladder tissue, the most common location for distemper inclusions outside of the lungs, was not available in any case. Inclusions were found in the reticulo-endothelial cells in the spleen in case 1, as they are in distemper. In case 5 they were probably present in bile duct epithelium, where they are often present in distemper. Further study is necessary before it can be concluded that there are no differences in organ distribution and cytotropism in the two conditions, but as far as our observations go, no differences have been found that are significant.

The occurrence in some cases of measles of giant cell pneumonia, identical both histologically and cytologically with Hecht's disease and pneumonia of distemper, raises the question of the interrelationship of these three conditions. It is possible, or perhaps probable, that Hecht's disease may be caused by the action of the measles virus in certain persons who, because of low resistance or for some other reason, do not develop the characteristic rash, Koplik spots, etc. If this is true, Hecht's disease should be regarded as identical with pneumonia produced by the virus of measles. On the other hand, certain considerations, particularly the chronicity of Hecht's disease and the usual absence of giant cell pneumonia or inclusions in fatal measles, suggest the possibility that two distinct viruses may be involved, only one of which causes a distemper-like morphological picture. Morphological

studies of the tissues of monkeys infected from human cases of measles should help to clarify the situation.

Distemper in Man

Evidence for the occurrence of distemper in man has not been reported as far as we have been able to learn, although there has been speculation concerning this possibility. The title of a paper by Taskin [10] suggests that canine distemper virus may produce inapparent infection in man. We have not been able to obtain the journal in which this paper was published.

The apparent enhancement of immunity to influenza by the distemper virus reported by Horsfall and Lennette [11] and the possible sparing effect on poliomyelitis noted by Dalldorf and Douglass [12] suggest a relationship between the distemper virus and other virus diseases of man. Eichorn and Pyle [13] found cross-immunity between distemper and influenza in ferrets, but this observation apparently has not been constant, since it has not subsequently been reported.

Relation to Vitamin A Deficiency

Chown [3] believed that the pathological changes in the lungs in giant cell pneumonia were a manifestation of vitamin A deficiency. This theory is of interest because of the fact that many of the clinical features of distemper resemble those of vitamin A deficiency. In ferrets and minks, hyperkeratinization of the skin of the paws and the occurrence of pustular lesions in the hair follicles, particularly around the nose and mouth, are definitely suggestive of vitamin A deficiency. In the bladders of ferrets, minks and foxes we have noted marked thickening and desquamation of the mucosal epithelium. This thickening is often most marked in the regions where inclusions are most numerous. Barondes [14] believed that some of the symptoms of distemper in dogs were suggestive of vitamin A deficiency. The possibility is suggested that the presence of the distemper virus in cells, with the consequent profound changes in cellular metabolism, may render the cells unable to utilize vitamin A. On the other hand, the metabolic disturbance caused by the virus in the cells (and manifested morphologically by the presence of the inclusions) may, by some other mechanism, bring about changes resembling those of vitamin A deficiency. This aspect of the problem is under investigation.

Epidemiological Possibilities

Distemper is primarily a disease of young animals. That the predilection for the young is not entirely explained on the basis of previ-

ous immunization of adults by subinfectious doses is indicated by the fact that in outbreaks of distemper on mink ranches, previously uninfected, the morbidity and mortality is much higher in young than in adult minks.[6]

Direct transmission of distemper from dogs to infants is a possibility which perhaps should be considered. In case 1 reported here, contact with animals was not a possibility, since the infant did not leave the hospital. The possibility that distemper infection may be latent in man should be investigated. With toxoplasmosis it is well known that infants may acquire fatal infection while *in utero* and in the absence of clinical manifestations of the disease in the mothers. Given the condition of latent infection in infants, other diseases such as measles, tuberculosis and syphilis might lower resistance and allow a distemper-like virus to produce pathological changes.

Relation to Other Virus Pneumonias of Infants

The inclusions described here are totally unlike those of "inclusion disease," described in lungs by Farber and Wolbach,[15] and others. In this condition, an instance of which was observed here recently, large cells are seen in the pulmonary alveoli, but these cells are rarely multinucleated. The nuclear inclusions are large and granular, filling and distending the nuclei.

The virus pneumonia of infants described by Goodpasture, Auerbach, Swanson and Cotter[16] does not show giant cells. Nuclear inclusions are granular and often fill the nuclei. Cytoplasmic inclusions do not occur. These features seem to distinguish this condition from giant cell pneumonia.

The virus pneumonia in infants described by Adams[17] is apparently unassociated with giant cell formation. Cytoplasmic inclusions occur in this condition. Certain of these inclusions resemble those seen in giant cell pneumonia (on the basis of published descriptions and illustrations and of our own observations on one section which we have studied). The cytoplasmic inclusions, however, are relatively small and relatively constant in size and shape, in contrast to the pleomorphism of the inclusions seen in giant cell pneumonia and in distemper. The absence of discoverable nuclear inclusions in carefully studied material from many cases also suggests that this is probably a different type of virus pneumonitis.

Two reports are found in the literature of pneumonia in measles associated with giant cells and cytoplasmic inclusions, but without nuclear inclusions. The case reported by Semsroth[18] was believed to be one of prodromal measles, although the clinical description was not

clearly that of measles. The case reported by Masugi and Minami[19] apparently was one of measles, but it is not clear whether giant cell pneumonia in these 2 cases was a manifestation of the activity of the virus of measles or of some other virus.

The occurrence of giant cells in lymphoid tissue early in the course of measles[20] is a well recognized pathological lesion of considerable interest. In the course of our studies, the possibility occurred to us that these giant cells and the giant cell pneumonia might be a manifestation of the action of the same virus. The only material available to us was a section of appendix from a case of prodromal measles. Careful study of the giant cells in the lymphoid tissue of this appendix revealed no cytoplasmic inclusions, and no definite nuclear inclusions, although an occasional suggestion of the presence of a nuclear inclusion was noted.

In distemper and in giant cell pneumonia, giant cells are absent from lymphoid tissue. Green and Evans,[8] however, have described giant cells with inclusions characteristic for distemper arising from cells of the adrenal medulla and from liver cells.

The occurrence of giant cells in tissues from patients with measles requires further study from the point of view of distribution and the presence of inclusion bodies. At present it is not clear whether they indicate the action of the measles virus or of some concomitant virus, such as, for example, that of giant cell pneumonia.

Summary and Conclusions

In a study of lung tissue from 5 cases of Hecht's giant cell pneumonia in infants, cytoplasmic and nuclear inclusions were found in 4 cases, while in a fifth case cytoplasmic inclusions alone were observed. The inclusions are found in bronchiolar epithelium, in alveolar lining cells and in giant cells arising from the latter two types of cells. Both cytoplasmic and nuclear inclusions are of the type associated with virus activity. The cytoplasmic inclusions are multiple, often vacuolated, and show marked variation in size and shape, while the nuclear inclusions are usually single, rarely filling more than one-third of the nucleus, and relatively constant in size and shape.

The cytological picture presented by this combination of cytoplasmic and nuclear inclusions is identical with that seen in distemper in dogs and other lower animals, and is quite unlike that seen in any other known type of virus infection. It is generally believed to be diagnostic of distemper in animals.

The virus of distemper in minks, ferrets and foxes causes an interstitial pneumonia in which giant cell formation from alveolar and

bronchiolar lining cells is a prominent feature. In several instances, giant cells of this type were found in large numbers, and the histological picture of giant cell pneumonia appeared to be duplicated in every detail. As in the cases of human giant cell pneumonia, the inclusions were located in bronchiolar and alveolar lining cells, and in the giant cells.

In sections from 2 of 6 fatal cases of clinically typical measles made available by Dr. Denton, an identical picture of giant cell pneumonia with nuclear and cytoplasmic inclusions was found. Possible interpretations of our observations are: (1) that giant cell pneumonia is a lesion caused by the measles virus, which may occur with or without the usual clinical manifestations of measles; and (2) that giant cell pneumonia is caused by another virus which may act independently or in association with the measles virus. In either case, the histological and cytological identity of the pulmonary lesions of giant cell pneumonia with those of canine distemper suggests that there is a close biological relationship between the two diseases.

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[*Illustrations follow*]

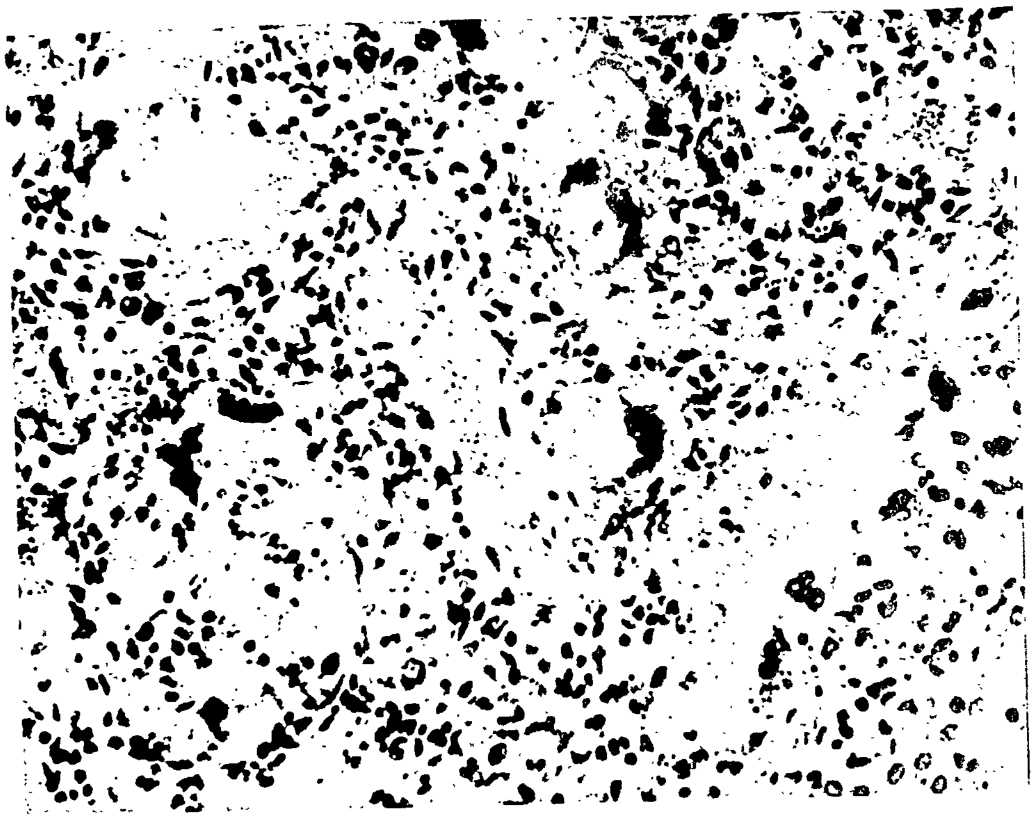
DESCRIPTION OF PLATES

PLATE 1

FIG. 1. Giant cell pneumonia in an infant (case 1), illustrating the distribution and appearance of the giant cells, most of which are derived from alveolar lining cells. Hematoxylin and eosin stain. × 280.

FIG. 2. Case 1. Syncytial multinucleated cell arising from the epithelium of a bronchus, and containing two inclusion bodies of medium size. (Arrows point to the inclusion bodies.) Hematoxylin and eosin stain. × 1000.

1



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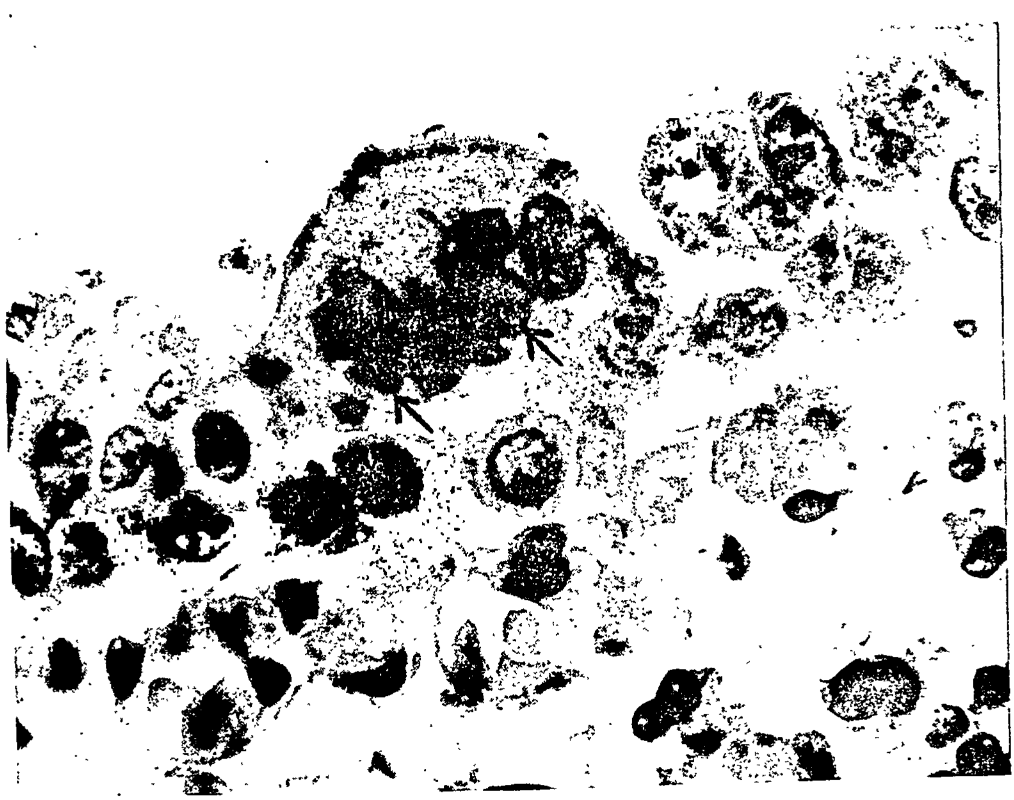

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Giant Cells in Pneumonitis

PLATE 3

FIG. 6. Distemper pneumonitis in a mink showing many giant cells arising from alveolar lining cells. This picture is similar to that seen in giant cell pneumonia (Fig. 1). Hematoxylin and eosin stain. × 280.

FIG. 7. Bronchial epithelium from the lung of a mink. Numerous deeply stained cytoplasmic inclusions are shown, with marked variation in size. Hematoxylin and eosin stain. × 1000.

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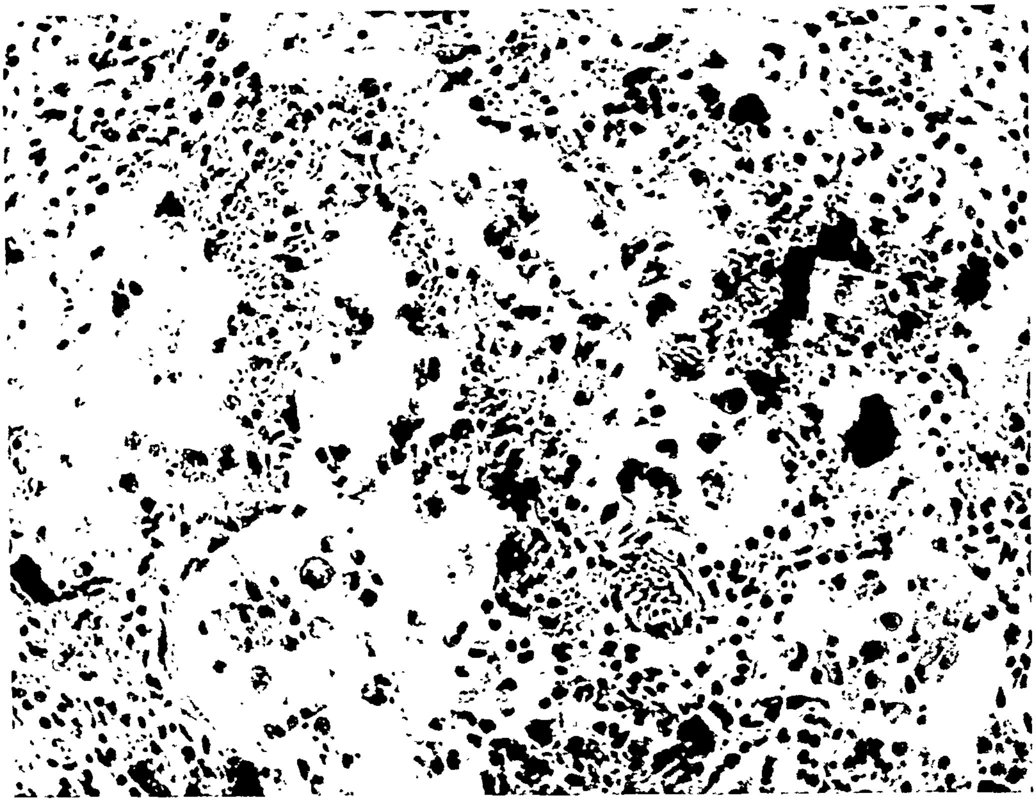

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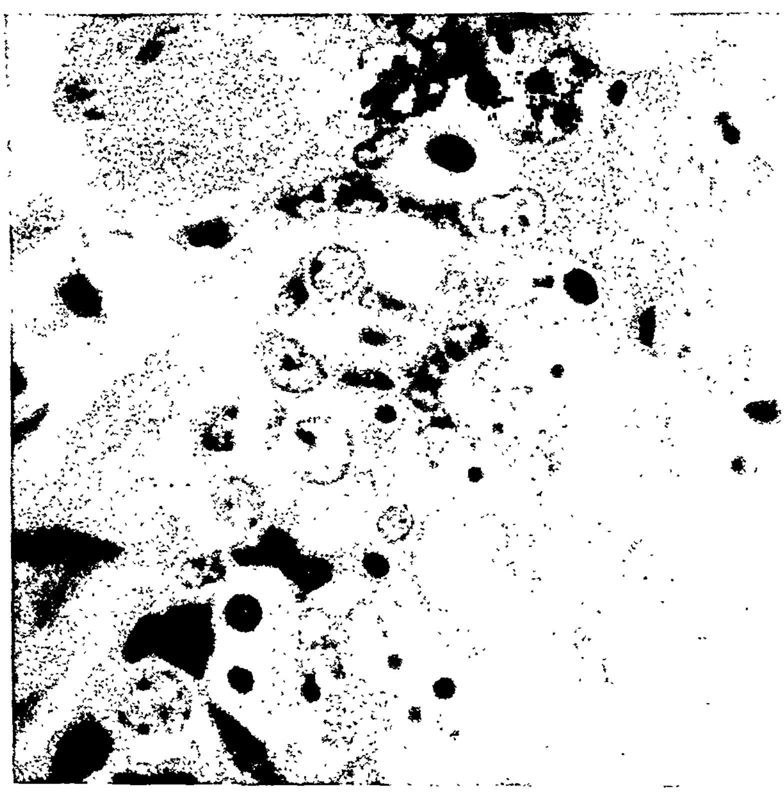


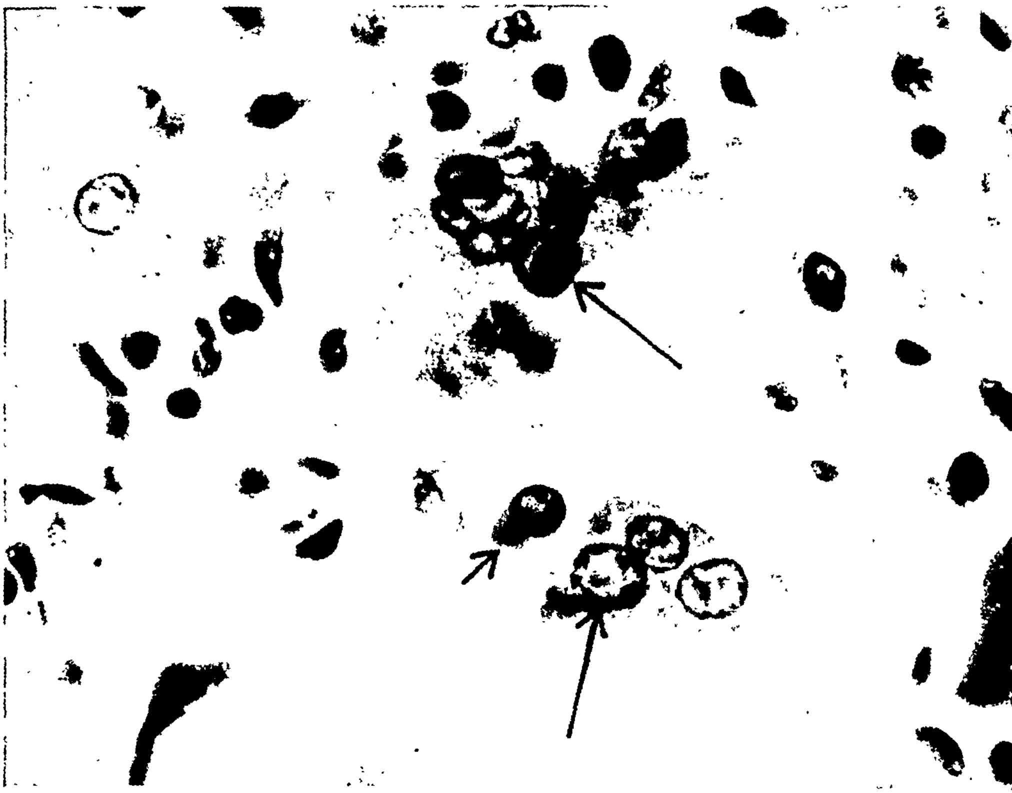
Pinkerton, Smiley and Anderson Giant Cells in Pneumonitis

PLATE 4

FIG. 8. Distemper pneumonitis in a mink. Two nuclear inclusions and one paranuclear cytoplasmic inclusion are seen in giant cells lining an alveolus. Other cytoplasmic inclusions are present, but not in focus. This picture, which is typical of distemper, is to be compared with Figures 4 and 5, showing a similar picture in the lung of an infant. Hematoxylin and eosin stain. × 1000.

FIG. 9. Distemper pneumonitis in a mink. Two giant cells arising from alveolar lining cells are shown. One of the giant cells contains three cytoplasmic inclusions. This picture is similar to that shown from the lung of an infant in Figure 3. Hematoxylin and eosin stain. × 1000.

8



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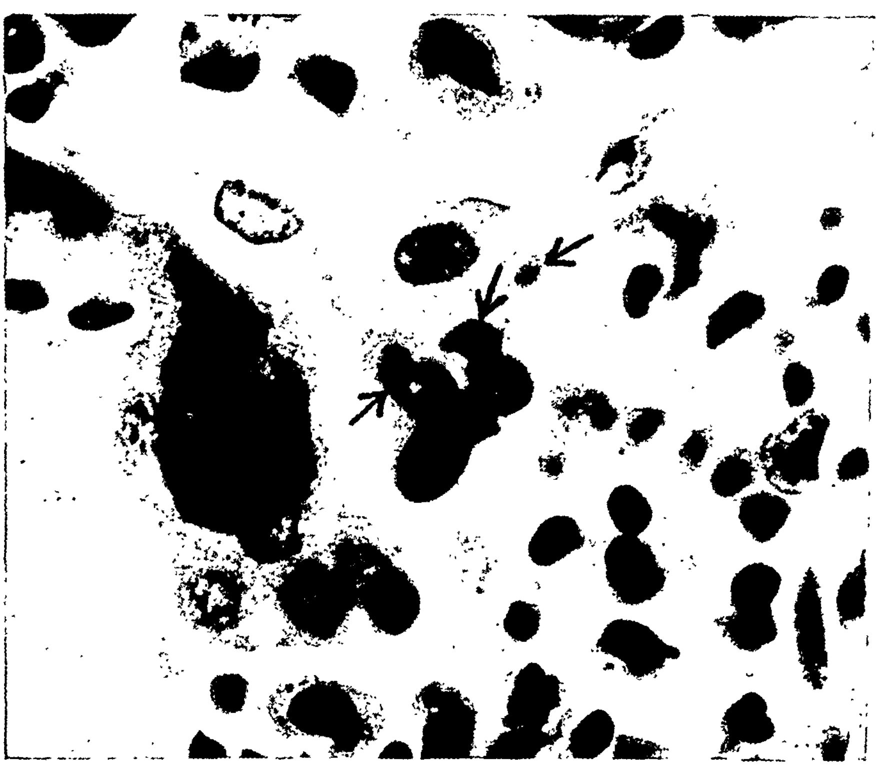

Pinkerton, Smiley and Anderson Giant Cells in Pneumonitis

PATHOLOGY OF SCLERODERMA, WITH SPECIAL REFERENCE TO THE CHANGES IN THE GASTROINTESTINAL TRACT *

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Numerous case reports and a few reviews on scleroderma have appeared in the literature and it does not seem justifiable to add other cases unless they present features which have not been previously emphasized. The following 2 cases are believed to be of interest because of lesions in the gastrointestinal tract that do not appear to have been described heretofore.

Scleroderma occurs in females twice as often as in males and most commonly in the fourth and fifth decades. Generalized and localized forms are recognized, the latter commonly known as morphea. The course of the disease may be rapidly or slowly progressive. Spontaneous recovery has been recorded, but is rare. The skin changes begin with edema and progress to induration and atrophy. In the latter stage the integument is of satiny smoothness and is tightly stretched over the bony prominences. Increased pigmentation of both exposed and unexposed parts of the body is often present. Pigmentation of the mucous membrane is practically never seen. Raynaud's syndrome may precede the sclerodermal lesions. Pain and a sensation of stiffness in the joints and muscles are common complaints and may be followed by joint and tendon contractures which are apt to incapacitate the patient. Sclerodactylia, ulceration of the skin, and osteoporosis of the bones of the extremities are common. Clubbing of the extremities has likewise been noted.

That scleroderma is not a disease of the skin alone but a generalized process has been realized for some time. Hektoen,[1] in 1897, described atrophy of the thyroid gland with endarteritis and hyperchromatophilia of the pituitary body, myocardial hypertrophy, and interstitial myocarditis. He attributed only the changes in thyroid and pituitary glands to scleroderma. Later, Matsui [2] reviewed the clinical and pathologic findings in 6 cases and emphasized the vascular lesions that occur in various localities.

The following case reports describe the clinical findings in our 2 patients:

Case 1

H. B., a housewife, 36 years old, of Polish extraction, was admitted to Goldwater Memorial Hospital on December 18, 1941, complaining of thickening of the

* Received for publication, February 4, 1944.

skin, pain on walking and poor appetite of about 15 months' duration. She stated that her mother had had similar skin lesions at approximately the same age, but had recovered completely. The patient had had the usual childhood illnesses without sequelae and had married at 26 years. In the next 5 years she had borne 3 children. In 1939, 2 years after the birth of her last child, when she was 33 years of age, she noted the presence of abdominal striae for the first time. These became more pronounced in the following year, when she experienced mild pains in the joints of the hands and feet, extending eventually to the knees and shoulders. During this period the patient felt cold, her appetite was poor and she lost 26 lbs. The skin over the extremities became swollen and movement was difficult.

On September 25, 1941, she had been admitted to the Presbyterian Hospital. At that time she was well developed and well nourished, the skin was deeply tanned, cool and dry, and smoothness and thickness of the skin were noticeable over the face, the anterior part of the chest and over the hands and feet. All the joints of the fingers were contracted and clubbing was present. She was unable to lift the right arm above the head. There was slight pitting edema of the legs and thighs. The abdominal striae were now prominent. The blood pressure was 110/70 mm. Hg. The patient was placed on a salt-free diet for 1 week, during which time the serum carbon dioxide, chlorides, calcium, sodium and nonprotein nitrogen values were within normal limits. Except for this interim, she remained on a high caloric, high vitamin diet. Medication included brewers' yeast, padutin,* crude liver extract, nicotinic acid, depropanex,† vitamin E and pancreatin. Physiotherapy was also employed. During her stay of 11 weeks she was seen by several ophthalmologists because of lateral nystagmus. This was attributed to congenital optic atrophy of unknown cause.

She was transferred to Goldwater Memorial Hospital in an unimproved condition on December 18, 1941. During the following year there was little change except for an additional loss of 15 lbs. in weight. She received 0.1 gm. of dry thyroid gland every day for several weeks. The skin lesions were slowly progressive. In November, 1942, she was forced to remain in bed because of weakness and stiffness of her extremities. In December, 1942, the patient complained of abdominal cramps for the first time. By April, 1943, this had become her predominant complaint and was associated with periods of nausea, vomiting and constipation. The patient was transferred to the surgical service on July 10, 1943, because of signs of intestinal obstruction. Many attempts to pass a Miller-Abbott tube were unsuccessful. Wangensteen drainage with a Levine tube was continued for 2 weeks but the patient became dehydrated. As soon as oral feeding was resumed, vomiting ensued. Occasionally this was induced by the patient herself for the relief of symptoms. Temperature ranged between 97° and 100.2° F. The blood pressure never rose above 112/80. She became gradually weaker and died on September 21, 1943. Necropsy was performed 26 hours post-mortem.

Gross Examination

The body was that of an emaciated white female, 36 years old. The scalp was freely movable, its hair dry, thin and covered by fine bran-like deposits. The skin of the face was smooth, shiny and tightly stretched across the underlying skeletal framework. The neck was long, thin and deeply tanned, the integument immobile. The abdomi-

* Padutin is a pancreatic tissue extract marketed by Winthrop Chemical Co., Inc., New York, N. Y.

† Depropanex is a pancreatic tissue extract marketed by Sharpe & Dohme, Philadelphia, Pa.

nal striae were white and contrasted strongly with the surrounding dark brown skin. The skin over the surface of the abdomen was desquamated in scales. Both the ventral and dorsal aspects of the trunk were light brownish or brownish yellow, the ventral surface being more deeply and extensively pigmented than the dorsal. The most intensely pigmented areas commenced at the lower third of the forearms and extended to the fingertips. Similar pigmentary altera-

	BLOOD	URINE	BLOOD CHEMISTRY VALUES	X-RAY	MISC.
Presbyterian Hospital 9/25/41 to 12/16/41	RBC 3,700,000 Hgb. 60% WBC 6,900 P. 80% L. 14% M. 5% E. 1%	Record not available	Phosphatase 5.1 B. U. Phos. 3.5mgm.% Ca. 9.7 " " NPN 22 " " Total cholest. 155 " " Free " 49 " " Esters " 106 " "	Trophic changes typical of scleroderma hands and feet. Normal sella turcica. Flat plate of abdomen normal.	B.M.R.-1 0 Stool normal. E.K.G. normal.
Coldwater Memorial Hospital Admission 12/19/41		s.g. 1.022 alb. 1+ occ. WBC	Urea N 9.1mgm.%		
7/12/43			Urea N. 32mgm.% Chlorides (as NaCl) 528 " " Total prot. 7.38 " " Alb. 4.8 " " Glob. 2.5 " "	G. I. Series: Periduodenal adhesion. Indirect evidence of obst. lesion at duodenal jejunal flexure. Barium enema: Generalized dilatation of entire colon without evid. of obst. lesion. Chest: Heart not enl.	B.M.R.+6 E.K.G.1° ht. block PR .24sec Right bundle branch block. V.P C., L.A.D.
9/20/43	RBC 3,200,000 Hgb. 77% WBC 10,100 P. 88% L. 8% M. 4% E.S.R. 51 mm/hr.	s.g. 1.013 alb. 4+ WBC 8/HPF RBC 3/HPF No casts	Urea N. 35 mgm.%	Heart enlarged to left. No pleural effusion. No infiltration or consolidation. Moderate congestion.	E.K.G. same as 7/12/43

Text-Fig. 1. Table I presenting laboratory data for case 1.

tions were present in the lower extremities, and the tissue covering the dorsal aspects of the feet was edematous, pitting on pressure. No pigmentary changes were present in the visible mucous membranes. The upper and lower extremities were in a position of semiflexion because of contraction of the biceps and hamstring tendons. The muscle tissues of all extremities were extremely atrophied and the skin covering them was tense. The nail beds were broadened to almost twice their normal width, and the nails were convex. There were many decubiti over the dorsal surface of the trunk, one of which had penetrated the sacrum and was filled with blood. Small encrusted areas of ulceration lay over the interphalangeal joints, which were flexed. Free joint motion was secured when linear incisions were made into the skin covering

the dorsum of the toes. The joint capsules appeared to be normal. Section of the skin covering the palms and soles revealed almost complete absence of fat pads.

The breasts and intercostal muscles were markedly atrophied. On opening the chest each pleural cavity contained 300 cc. of clear serous fluid. The lungs together weighed 655 gm., and were feathery and crepitant throughout except at the extreme bases where they were deeply congested.

On opening the pericardium 15 cc. of cloudy, straw-colored fluid escaped. The pericardial sac was obliterated by adhesions over an area corresponding to about two-thirds of the cardiac surface. On stripping these adhesions the surface of the heart presented a granular appearance (Fig. 1). The heart weighed 240 gm. All valves were thin and delicate. The endocardium of the left ventricle, especially near the apex, was opaque and thickened. The myocardium was firm, brownish, and presented a wax-like appearance. The iodine test for amyloid was negative. On section the myocardium was found to be streaked by fine grayish bands of tissue which seemed to enter both from the epicardial and endocardial surfaces. These streaks were present with noticeable frequency in the interventricular septum in an area corresponding to the position of the conduction system.

The muscle tissues of the anterior abdominal wall were thin, and the overlying skin was thickened but freely movable. The peritoneum enclosed 700 cc. of straw-colored fluid. The visceral layer was thin, gray, smooth and glistening. The parietal layer was thickened. The gastrointestinal tract was of normal caliber throughout. No mucosal lesions were noted but the mucosa of the jejunum and ileum was deep brown.

The kidneys together weighed 225 gm. The surfaces were smooth. The cortices measured 0.8 cm. The cortical striations were distinct. A few glomeruli could be distinguished as pinpoint-sized gray dots. The medullae were congested.

The thyroid was small and on section presented a light brown surface streaked with whitish lines.

The remaining organs, including the parathyroids, pituitary body, adrenals, brain and cord, liver, spleen, pancreas, the bones of the skull, the ribs and vertebrae, showed nothing of note in the present connection.

Histologic Examination

Skin. The surface of the skin was covered by deposits of keratinized material. The epidermis was atrophied, the rete pegs slender and short. In the cells of the basal layer, pigment was present in greatly increased

quantities. The derma was thickened and edematous (Fig. 2). The subcutaneous fat was divided into lobules of irregular size by wide bands of connective tissue. The remaining fat showed the changes incident to serous atrophy and was diffusely infiltrated by lymphocytes and plasma cells. Occasionally small deposits of calcium were apparent in the collagenous fibers. The various appendages of the skin were surrounded by bands of coarse connective tissue. Similar bands surrounded many of the nerve fibers and blood vessels as well as the corpuscles of Vater and Meissner. The sweat glands, although atrophic, showed no constriction by collagenous tissue. The arrectores pilorum were hypertrophied and only occasionally encroached on by fibrous tissue. The lumina of the medium-sized arteries were narrowed by concentric layers of fibrous tissue in the intima. The internal elastica was often fragmented. Less conspicuous alterations were present in the form of fibrous replacement and hyalinization of the media. The smaller arteries and the arterioles, mainly in the areas of serous atrophy of fat, were thickened and presented a smudged appearance due to fibrinoid necrosis. Occasionally the lumen was obstructed by swollen endothelial cells. No thrombi were observed. In general, where the connective tissue fibers of the skin were coarsest, the elastic fibers were scarce. In the subcutaneous fat there was a fringe of fibrils along the borders of the collagenous bands.

The diaphragm, the intercostal muscles and those of the abdominal wall were atrophic. The striations were well preserved.

Thyroid. The thyroid was richly penetrated by fibrous bands which appeared to originate in the sclerotic capsule. At the periphery the organ was diffusely infiltrated by lymphocytes. The acini were small, uniform in size and filled with colloid. The cuboidal epithelial lining was well preserved. The blood vessels were embedded in thick connective tissue bands and the lumina of many of them were narrowed.

Heart. In the epicardium were bands of fibrous tissue together with fresh fibrinous deposits, polynuclear leukocytes, lymphocytes and plasma cells (Fig. 3). The parietal pericardium was similarly involved. Bacteriologic culture of the pericardium was sterile. The myocardium was traversed by connective tissue bands, many of which were perivascular. Almost all of the medium-sized and smaller arteries showed eccentrically thickened walls and narrowed lumina due to intimal fibrous tissue proliferation. There were ill-defined focal areas of fibrosis, in the midst of which were a few fairly well preserved muscle fibers. These areas were well vascularized and cellular (Fig. 4). The endocardium was tremendously thickened by overgrowth of fibrous tissue which practically replaced all of the trabeculae carneae, only a

few degenerate muscle fibers remaining. This tissue was richly infiltrated by polymorphonuclear leukocytes, lymphocytes, and plasma cells. Clumps of light brown, iron-free pigment were scattered through the affected areas (Fig. 5).

Lungs. The walls of the alveolar septa were thin and the alveoli contained serous fluid and desquamated cells. The periphery of the lower lobes was extensively fibrosed. The fibrous tissue was richly vascularized and was especially abundant around the blood vessels and smaller bronchi. In these areas many of the alveoli were lined with tall columnar, mucus-containing cells which were continuous with those of the terminal bronchioles.

Gastrointestinal Tract. Throughout its entire extent the mucosal layer of the esophagus was replaced by fibrillar, acellular tissue to which a few red blood cells were adherent. Beneath, and sometimes mingled with it, were bands of muscle tissue representing the remains of the inner longitudinal layer. Many of these fibers showed fibrinoid degeneration but no cellular infiltration between them. The submucosa was composed of dense collagenous tissue. The circular muscle was almost completely replaced by fibrous tissue, isolated fibers persisting as islands lying in edematous fibrosed and vascularized areas. The longitudinal fibers were few in number, thin and atrophic. The myenteric plexuses of Auerbach were carefully studied but no deviation from the normal was found. The serosal layer was edematous and fibrotic (Figs. 6 and 7). Except for some edema and atrophy of the muscularis, the stomach appeared to be well preserved. The mucosa of the jejunum showed diffuse lymphocytic infiltration. The villi enclosed masses of hemosiderin. The submucosa contained many dense collagen fibrils but was not thickened. Both muscle layers were atrophied. In many places the muscle layer was so extensively replaced by bands of fibrous tissue that the mucosal surface was brought into close approximation with the serosa. The mucosa and submucosa of the appendix were hyalinized and the lumen reduced to a slit-like aperture. The vessels were greatly thickened and hyalinized. The muscularis was atrophic. The changes in the colon were similar to those in the small intestine. The lesions described were patchy in character. The vascular changes in the gastrointestinal tract were slight but the serosa was fibrotic and thickened throughout (Figs. 8 and 9).

Mesentery. A thick layer of connective tissue covered the peritoneal surface. The nerves and vessels were encased in fibrous tissue and the fat was reduced. One medium-sized artery showed a narrowed lumen due to intimal proliferation and edema, and infiltration by lymphocytes (Fig. 10).

Kidneys. The majority of the glomeruli were damaged. A few were hyalinized, others necrotic, while still others showed the so-called "wire-loop" appearance. Often the glomeruli and Bowman's space contained polymorphonuclear leukocytes and red cells which extended into the proximal convoluted tubules. The predominating vascular lesions were in the arteries of small caliber and consisted of: (1) intimal edema and proliferation; (2) widespread necrosis of the muscularis or of the entire wall; and (3) hyalinization with narrowing of the lumen. Combinations of these lesions might be seen in the same vessel. The walls of the arteries and the edematous interstitial tissues were infiltrated by polymorphonuclear leukocytes, lymphocytes and plasma cells. The tubules contained many casts of different types and the tubular epithelium was crowded with hyaline droplets. The walls of the pelves were markedly fibrotic and the vessels showed much the same lesions as those in the parenchyma although there were, in addition, perivascular connective tissue bands.

Cervix. The uterine cervix showed an area of erosion. The lamina propria was diffusely thickened at the mucocutaneous junction by coarse and heavily vascularized connective tissue. The thin-walled vascular channels were surrounded by bands of fibrous tissue. Deep in the cervical wall the medium-sized arteries showed the proliferative endarterial changes described elsewhere. Bands of perivascular connective tissue also outlined the thick-walled vessels.

Histologic examination of the remaining organs, including the parathyroids, adrenals and pituitary body, liver, spleen, pancreas, fundus uteri and ovaries, showed nothing of note in the present connection.

Anatomic diagnoses included: generalized scleroderma; acute fibrinous and chronic pericarditis; fibrosis of endocardium and myocardium; pleural effusion, bilateral; ascites; edema of feet; diffuse glomerular and arterial lesions of the kidneys; muscular atrophy of the gastrointestinal tract; degeneration of esophageal mucosa; minimal pulmonary fibrosis; multiple decubiti; muscular atrophy; atrophy of thyroid; partial degeneration of optic nerve, chiasm and tracts.

Case 2

W. K., a car draftsman, 56 years old, presented himself at the Vascular Clinic of the New York Post-Graduate Hospital on May 12, 1942. He said that he had been in excellent health until August 21, 1941, when, following the ingestion of frankfurters, he experienced an episode of diarrhea, puffiness of the face and ankles, and muscle pains. The white blood count at the time was said to be elevated and to contain 8 per cent eosinophils. No muscle tissue was removed for biopsy. Skin tests for trichinosis were negative. In December, 1941, the patient noticed that his hands and feet were often stiff and cold. This condition became progressively worse so that in January, 1942, he was forced to give up work. Two months later

the stiffness had progressed to involve the skin of the entire body. At this time a piece of skin was removed for biopsy and was reported as showing "some predominance of fibrous tissue." At the Vascular Clinic it was noted that the skin over the entire body was taut, dry and deeply pigmented. Joint motion was limited because of contractures of the skin. The blood pressure was 110/70 mm. Hg, the peripheral pulses were of good quality, and oscillometric readings were normal.

Treatment included mecholyl * iontophoresis, depropanex and dihydrotachysterol.

The patient attended the clinic until June 16, 1942, when he was admitted to the Post-Graduate Hospital and remained until July 10, 1942. During this period he complained for the first time of difficulty of swallowing and of a dull, midepigastric pain which had no relation to meals. He also developed a cough productive of white mucoid sputum.

	BLOOD	URINE	BLOOD CHEMISTRY VALUES	X-RAY	MISC.
August, 1941 to May, 1941	RBC 4,490,000 Hgb. 86% WBC 15,450 P. 82% L. 14% M. 2% E. 2%	Routine — normal. No arsenic in 24 hr. spec.	NPN 26.5 mgm.% Creat. 1.16 " "		
Post Graduate Hospital 5/12/42 to 7/12/42	Wassermann — negative. RBC 4,780,000 Hgb. 72% WBC 17,000 P. 68% L. 21% M. 8% E. 12% B. 1%	Several — normal. Addis count: Vol. 190 c. c. Ph. 5.3 Casts 74,100 RBC 47,550 WBC 110,200 Urinary steroids 277 mgm., 24 hr. 17 ketosteroids 4.8 mgms.	Phos. 6.2 mgm.% Ca. 10.2 " " Urea N. 10.5 " " NPN 29.5 " " Chlorides (as NaCl) 485 " " Glucose 80 " " Cholest. 195 " " " esters 115 " "	Chest: Moderate ht. enlargement—mainly left vent. Mod. hilar root branch thickening mainly toward bases. Sl. pleuritic thickening left costo-phrenic sinus.	Vit. C. saturation normal. B.M.R.+6
7/9/42		Routine — faint tract alb., 1-2 hyalin/HPF 1-3 gran./HPF			
Coldwater Memorial Hospital 7/10/42 to 7/17/42		Normal	Urea N. 43 mgm.%	Bilateral pleural effusion ext. into fissures and a sl. generalized enlargement of heart.	E.K.G.—L.A.D. Low voltage in lead II P. R. interval .16 sec.

Text-Fig. 2. Table II presenting laboratory data for case 2.

The physical findings were unchanged. He was comfortable when lying in bed, and fairly well nourished despite the loss of an unknown amount of weight. The heart was not enlarged to percussion. There was a soft apical systolic murmur and the sounds were of good quality. The lungs were clear; the abdomen was difficult to palpate because of the density and inflexibility of the skin. The left epididymis was slightly thickened. There was no edema. The skin and joints were as previously described. The blood pressure varied between 120/80 and 90/70 mm. Hg. The temperature ranged from 99.2° to 100.8° F.

When the patient entered Goldwater Memorial Hospital on July 10, 1942, his blood pressure had risen to 158/100, and he died suddenly 1 week after admission.

* A proprietary name for acetyl-β-methylcholine chloride.

Gross Examination

The body was that of a fairly well developed and nourished white male, 56 years of age. The skin over the shoulders, forearms, hands, abdomen and feet was deeply pigmented and inelastic. Almost the entire body covering was affected. The fingers and toes were held in semiflexion by the stretched, unyielding integument. On section the skin was thickened and the subcutaneous fat traversed by bands of white fibrous tissue. The intercostal muscles in the second left space were extremely thin and the pleura could be seen beneath the fascia.

The right pleura enclosed 250 cc. of amber fluid; the left, 150 cc. The left lung weighed 470 gm. The pleural surface was opaque. The lung felt airless. On section it cut with great resistance. The substance of the lung was increased by fibrous tissue. The right lung weighed 640 gm. and presented essentially the same naked-eye appearances as the left. The increased density of the lungs was more striking in the lower two-thirds than in the upper one-third, which was moderately congested and edematous.

The pericardium contained 50 cc. of amber colored fluid. The heart weighed 350 gm. The epicardium was thick and opaque. The parietal pericardium was similarly thickened. The endocardium of the right side of the heart was thin and glistening. The endocardium of the left ventricle was opaque and thickened, particularly on the posterior and septal walls. The myocardium was brown, flabby and showed ill-defined grayish areas. About 2 cm. from its origin the caliber of the anterior descending branch of the left coronary artery was slightly reduced because of subintimal plaques.

No lesions were noted in the esophagus.

On opening the abdomen about 100 cc. of amber fluid was found. The musculature was thin and flabby. Both layers of the peritoneum were thick and white, and numerous adhesions bound the viscera together.

The intestine was of normal caliber. The stomach was dilated.

The right kidney weighed 170 gm.; the left, 180 gm. The capsules stripped with difficulty leaving irregular granular surfaces in which yellowish nodules projected against a dark purple-red background. The cortices were narrowed, the striations indistinct.

The thyroid was small, light brown and showed almost no colloid on section.

The rest of the organs revealed nothing worthy of note in the present connection.

Histologic Examination

Skin. The epithelium was atrophic. Small amounts of pigment were present in the basal layer. The derma showed clumps of extracellular melanin and was edematous and thickened. The subcutaneous connective tissue was coarse, avascular and contained only a few fat deposits. The walls of the smaller arteries and of the arterioles were edematous and surrounded by bands of fibrous tissue. A few areas of necrotizing arteritis were present in them. The hair follicles, the arrectores pilorum and the sebaceous glands were buried in dense constricting bands of connective tissue. The sweat glands were atrophic, but otherwise unchanged. In the scalp the arrectores pilorum were hypertrophic. Many of the medium-sized arteries and the arterioles in the deeper layer of the derma were occluded by fibrotic proliferation and edema of the walls. In some arteries all vascular coats had been penetrated by red blood cells from the lumen.

Striated Muscle. Sections from muscles in various parts of the body showed atrophy of the individual fibers with retention of the striations and some proliferation of sarcolemmic nuclei. Occasionally the fibers were swollen and fragmented and the nuclei were pyknotic. No Trichinella were seen (Fig. 11).

Heart. The epicardium was fibrous throughout and, over the surface of the right ventricle, hyalinized. Cellular infiltration of the epicardium varied in degree, and was predominantly lymphocytic in character. In the myocardium were ill-defined areas of fibrosis, many of which were continuous with the endocardium while others occurred in the midst of relatively well preserved muscle tissue. The centers of the latter lesions were well vascularized by minute blood channels surrounded by lymphocytes. The endocardium of the left ventricle showed a few patchy areas of thickening. The smaller arteries were encircled by fibrous bands, but only a few of them were edematous with their lumina narrowed.

Lungs. Sections of all lobes revealed very marked increase in fibrous tissue. The alveoli were widely replaced by richly vascularized and hemorrhagic fibrous tissue. The pleural surfaces were extensively fibrotic. The larger arteries of the parenchyma showed only a small amount of subendothelial fibrosis. Almost without exception the walls of the smaller arteries and of the arterioles were greatly thickened by edematous fibrous tissue and swollen endothelial cells. In many instances the lumina of the arterioles were almost completely obliterated (Fig. 12). Section of the right lower lobe showed an acute pneumonic process.

Thyroid. Except for a solitary, circumscribed, adenomatous nodule

the thyroid was poor in colloid and was diffusely infiltrated by lymphocytes which, in places, were so dense as to obscure the parenchyma. There were several small nodules composed of Hürthle cells. Surrounding these areas the parenchyma was largely replaced by tall proliferating epithelial cells and lymphocytes. Intravesicular papillary projections were rare. The greater part of the parenchyma was fibrosed.

Gastrointestinal Tract. The entire stomach wall was less than half the normal thickness. The mucosa was intact, the muscularis mucosae thin and in many places replaced by fibrous tissue, and the submucosa was composed largely of coarse connective tissue fibers. The muscle layers were atrophic. The serosa was slightly thickened.

The submucosa of the intestines contained numerous coarse connective tissue fibers. The muscularis mucosae was occasionally thinned. Marked edema separated the muscle layers. In many places the muscle layer was replaced by fibrous bands in both the circular and longitudinal layers. The plexuses of Auerbach appeared to be large in contrast to the atrophic muscle.

The peritoneum was thickened, its blood vessels dilated and congested. An infiltration of lymphocytes extended from the peritoneum into the surrounding fat tissues. The intima of some of the larger arteries was thickened. The arterioles shared the edema of the surrounding tissues.

Kidneys. The glomeruli were small. Many were congested, others bloodless. Some showed areas of fibrinoid necrosis, others had thickened capillary walls; a few were hyalinized. In some of the loops the limiting membrane was hyalinized; in others the capillaries were dilated and contained large numbers of polynuclear leukocytes. In some instances the glomerular spaces enclosed red blood cells extending into the proximal convoluted tubules. The interstitial tissue was increased, especially in the region of the glomerular capsule where it was infiltrated with polynuclear leukocytes, lymphocytes and eosinophilic myelocytes. The tubular epithelium was poorly preserved, most of the cytoplasm being granular. In the tubules were many casts of various types. In practically every medium-sized and small artery advanced fibrinoid necrosis involved the entire wall. A few polynuclear leukocytes and lymphocytes were present in such areas. In some vessels the lumina were obliterated by the necrotizing process, while in others edema and separation of the collagenous fibers produced narrowing of the lumen. In some, necrosis was confined to the muscular coat (Figs. 13 and 14). The renal pelves were thickened by coarse connective tissue fibers beneath intact epithelium.

Prostate. Sections showed adenomatoid hyperplasia. In addition, the walls of the vessels at the periphery were fibrotic. In several areas the smooth muscle fibers were atrophic.

Anatomic diagnoses included: generalized scleroderma; necrotizing and proliferative arteritis; pulmonary fibrosis; hydrothorax, bilateral; dilatation of heart; coronary sclerosis, mild; myocardial fibrosis; chronic passive congestion of liver; diffuse glomerular and arterial lesions of kidney; atrophy of thyroid; atrophy of intercostal muscles, and muscular atrophy of gastrointestinal tract.

Discussion

The cause of scleroderma has been variously ascribed to infectious and toxic, neurogenic, endocrine and vascular disturbances. The interrelationship of scleroderma with dermatomyositis, disseminated lupus erythematosis, the Libman-Sacks syndrome and polyarteritis nodosa is adequately discussed by Banks.[3] MacCallum[4] and Klemperer, Pollack and Baehr[5] have suggested an alteration in the colloidal system of the connective tissue as the factor which offers the most promising field of investigation.

In case 1 of this report the predominant complaints for the last 5 months of the patient's life were referred to the gastrointestinal tract. Clinical and roentgenologic evidence seemed to point to obstruction of, and disturbance in, the pattern of the mucosa of the small intestine. Barium enemas indicated irregular muscular contractions and diffuse dilatation of the colon. In case 2, late in the course of the disease, the patient developed dysphagia and complained of midepigastric pain.

The histologic changes were impressive in the gastrointestinal tract and appeared to explain the clinical findings in this report. The mucosa of the esophagus in case 1 was completely replaced by fibrinoid material, the submucosa was sclerotic, the muscle layers atrophic and extremely fibrosed. Rake[6] also has called attention to loss of the esophageal mucosa in scleroderma. In his case the submucosa was infiltrated by lymphocytes and polynuclear leukocytes so that the inflammatory nature of the lesion could not be denied. Recently, Lindsay, Templeton and Rothman[7] have recorded 3 cases of stricture of the esophagus in scleroderma in which tissue removed for biopsy revealed ulceration of the superficial epithelium and dense infiltration of the immediately surrounding tissues by neutrophils, eosinophils and plasma cells. In one of the cases here recorded there was no cellular infiltration in spite of the fact that the patient had a Levine tube in position for long periods; therefore it seems that the lesion of the esophagus should be attributed to scleroderma and not to trauma produced by the tube.

Thickening of the submucosa, and fibrosis and atrophy of the muscularis have been noted by many observers.[7-10] Cases have been recorded with esophagoscopic and roentgenologic evidence of obstruction at the lower end of the esophagus, but none of these has been confirmed by necropsy. It would appear that such lesions are due to spasm and that, as the muscle atrophies, spasm ceases. This may account for the improvement which has been noted after dilatation and sympathectomy. The remainder of the gastrointestinal tract in both cases here recorded showed extensive, although patchy, changes. Thickening of the submucosa was not a particularly prominent feature in either case. Diffuse dilatation of the colon demonstrated by barium enema in case 1 may be explained by deficiency of smooth muscle. Matsui[2] mentioned hypertrophy of the muscularis mucosae but atrophy of the muscularis propria. In my cases either no change in the muscularis mucosae or atrophy was observed. Edema was present in the muscularis adjacent to areas of muscle atrophy. This latter change is in accord with the general pattern of the disease as it is seen in the skin in the form of edema, induration and atrophy. Vascular alterations in the gastrointestinal tract were not conspicuous.

Lesions of the gastrointestinal tract in scleroderma, with the exception of those described in the esophagus, have received little attention. A third patient with diffuse scleroderma, who was under observation for 3 years in the Goldwater Memorial Hospital, developed signs of intestinal obstruction. Adhesions between intestinal loops were found at operation. No point of obstruction was determined but the small intestine was diffusely dilated. Death followed 12 hours later. Permission for necropsy was not obtained.

In case 1 of this report pericarditis was present and appeared to be both recent and old. According to Lewin and Heller,[11] pericarditis was present in 8 of 29 cases observed at necropsy. In view of the fact that some of these cases were associated with other conditions, notably tuberculosis and rheumatism, it is difficult to say whether the accompanying pericarditis can be attributed to scleroderma. In the cases here described the lesion in the pericardium presented hyalinization of collagenous tissue which was reconcilable with the lesions of scleroderma in other parts of the body. In case 1 the acute lesion overlying the chronic fibrous pericarditis was diffuse and too advanced to be included among those related changes which sometimes occur in uremia. In view of the fact that there were similar thickening and hyalinization of other serous membranes, particularly the peritoneum, it seems justifiable to include those in the pericardium as part of the disease, scleroderma, and not as manifestations of the uremic state.

The endocardial and vascular changes were more severe in case 1. The myocardial lesions were of like intensity. In both cases electrocardiograms showed left axis deviation and, in case 1, right bundle branch block. Clinically, neither patient showed any signs of cardiac insufficiency. Because most of the changes in the heart in scleroderma have been described in detail by Weiss, Stead, Warren and Bailey,[12] there is nothing to be added except to record the widespread vascular lesions observed in the medium-sized and small-sized arteries of the myocardium.

Although extensive pulmonary fibrosis was present in case 2, clinical signs were negligible, the patient having complained only of mild dyspnea on exertion. The anatomic lesions were out of all proportion to the clinical and roentgenologic findings. The vascular alterations in the lungs were more extensive than those found elsewhere. However, in case 1 the changes were confined to the periphery of the lungs at the bases, and were of slight degree. Even here the perivascular and peribronchial fibrosis seemed to represent sclerodermatous change. Linenthal and Talkov [13] have reported 3 cases with extensive changes in the lung, as revealed by roentgenologic examination together with clinical signs of pulmonary insufficiency and Raynaud's syndrome. Although fibrosis of the lung is frequently described in generalized scleroderma, practically no mention is ever made of pulmonary insufficiency.[2, 12, 14]

The thyroid was one of the first glands to be implicated in considering the pathogenesis of scleroderma. In case 1 the changes in the thyroid consisted of moderately increased fibrous tissue and proliferative lesions in the arteries. In case 2 the changes were more extensive and involved the architecture of the entire gland. Clinically, the patients showed no signs of disturbed thyroid function.

Parathyroids were available for examination only in case 1. They appeared to be normal, but it was noted that one of the branches of an artery supplying the gland showed proliferation in the intima. No changes were seen in the bones with the exception of osteoporosis of the fingers in case 1. In both cases the serum calcium and phosphorus were normal.

The pituitary body has been thought by some to be the seat of dysfunction in scleroderma, hyperchromatophilia and areas of necrosis having been described in the anterior lobe. In neither of our cases were changes observed other than those of post-mortem autolysis.

The adrenals have been incriminated in scleroderma largely because of bronzing of the skin. Unlike Addison's disease, pigmentation of the mucous membranes has not been mentioned. In the majority of cases

observed at necropsy the adrenals appeared to be normal. In the 2 cases reported here no structural alterations were noted in the adrenals although both patients exhibited rather intense pigment deposits in the skin. Neither patient had received x-ray therapy.

In both of the cases here recorded extensive renal lesions were found, especially in the medium-sized and smaller arteries. The glomerular lesions were similar to those observed in lupus erythematosis. Tubular alterations were severe. The renal pelves in both cases were thickened. The larger vessels in the peripelvic fat showed the same sort of perivascular fibrosis as that observed in other parts of the body. Compared with the histologic alterations, clinical signs and laboratory data indicating renal disease were slight.

Uterine atrophy has been noted by several observers. In case 1 no such change was found. On the other hand, the lamina propria of the vagina and the external cervical os shared in the generalized connective tissue thickening that occurred in the skin and elsewhere. The ovaries appeared to be normal.

Although it is difficult to estimate the amount of smooth muscle in the prostate, in case 2 it appeared to be atrophic. This may represent a sclerodermal lesion rather than an involutional change. Except for proliferative changes in the intima of the arteries the testes were normal.

Many studies have been made of scleroderma and its possible relationship to dermatomyositis.[3, 12] However, the borderline is not clear. In case 1 the changes in the skeletal muscles were slight and were attributable to atrophy from disuse. In the other case the muscle changes were somewhat more severe, but in neither case were infiltrations of lymphocytes present as in dermatomyositis, nor was there extensive muscle damage characteristic of that disease.

The changes in the skin were the same as those customarily encountered in scleroderma; namely, increase of connective tissue, atrophy of fat, edema, vascular lesions, calcinosis, atrophy of epithelium and increased pigmentation.

Changes in the central nervous system were studied extensively by Dr. Abner Wolf and were not considered noteworthy in the present connection.

Unfortunately, the sympathetic ganglia were not studied. The sympathetic plexuses in the gastrointestinal tract showed no structural alteration.

Whether lesions of the sympathetic system are responsible for the spasm exhibited roentgenologically in the gastrointestinal tract awaits

investigation. To date, the results of sympathectomy for Raynaud's syndrome in patients who develop scleroderma have been variable and not encouraging.

CONCLUSIONS

In two cases of generalized scleroderma endocardial and myocardial fibrosis, widespread vascular alterations, pulmonary fibrosis and severe kidney lesions were found, in addition to the usual dermal alterations. These changes were more severe than the clinical course of the disease and the laboratory data indicated.

Evidence is presented to include pericarditis as a manifestation of scleroderma.

Scleroderma is not a disease marked by connective tissue overgrowth alone, but also includes muscle degeneration and atrophy with or without connective tissue replacement. The severity of the muscle changes bears no direct relationship to the lesions of the vascular system.

The muscle atrophy throughout the gastrointestinal tract and replacement of the mucosa of the esophagus by fibrillar material are integral parts of the disease, scleroderma, and are related to some of the symptoms and signs observed during life.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

Plate 5

Fig. 1. Case 1. Heart with extensive pericarditis.

Fig. 2. Case 1. Skin of chest wall showing atrophic, deeply pigmented epidermis, and thickened derma and subcutaneous tissue. There is a connective tissue band about the atrophic hair follicles. $\times$ 160.

Fig. 3. Case 1. Epicardium with fibrinous exudate on surface, and fibrosis and cellular infiltration of the deeper layers. $\times$ 30.

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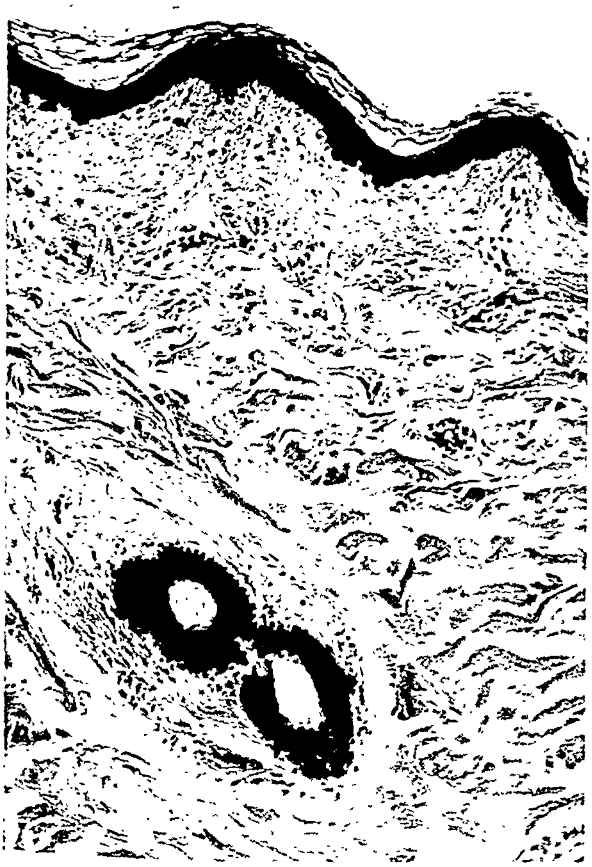

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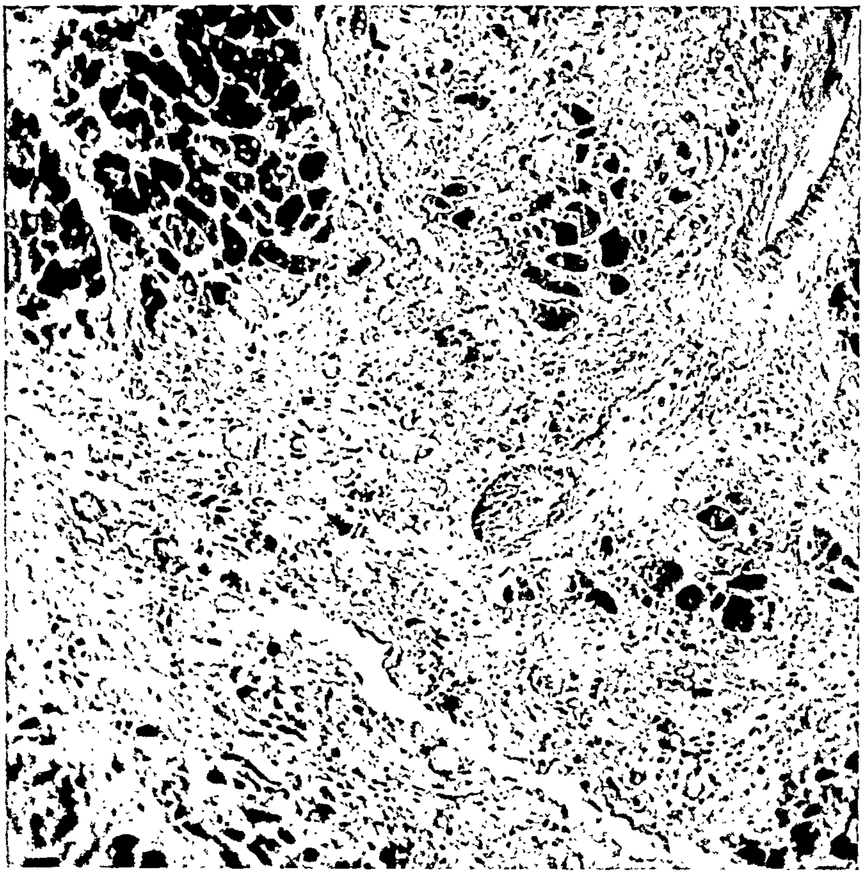
Bevans Scleroderma

PLATE 6

FIG. 4. Case 1. An area of myocardial fibrosis in which a vessel with thick, partially necrotic walls can be seen. × 172.

FIG. 5. Case 1. Endocardium with areas of thickening by fibrous tissue, which also extends into and partially replaces the muscle. Trichrome stain. × 31.

FIG. 6. Case 1. Esophagus with fibrillar material on mucosal surface, a thick fibrotic submucosa and extensive fibrous replacement of the muscle layers. Trichrome stain. × 31.



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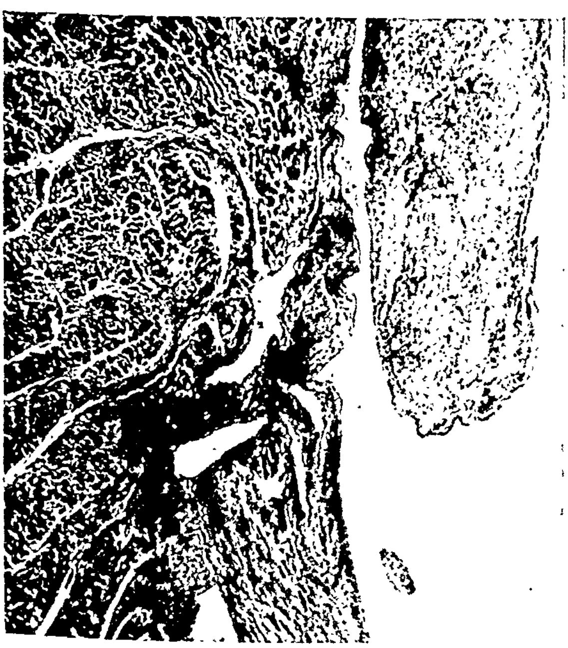

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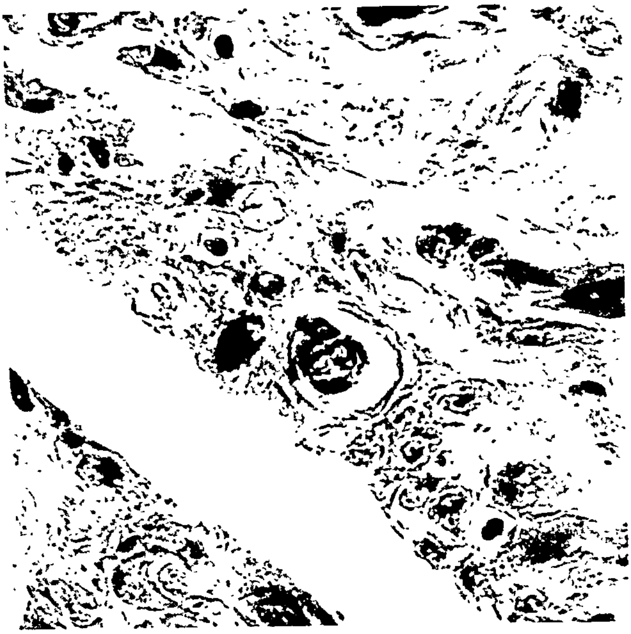
Bevans Scleroderma

PLATE 7

FIG. 7. Case 1. Higher magnification of muscularis of esophagus showing atrophic muscle fibers. Trichrome stain. $\times$ 972.

FIG. 8. Case 1. Section of the intestine showing loss of the longitudinal muscle at the left, and edema and atrophy of circular muscle. Trichrome stain. $\times$ 38.

FIG. 9. Case 1. Another section of the intestine with only a narrow band of fibrous tissue joining mucosa and fibrotic, thickened serosa. Trichrome stain. $\times$ 30.



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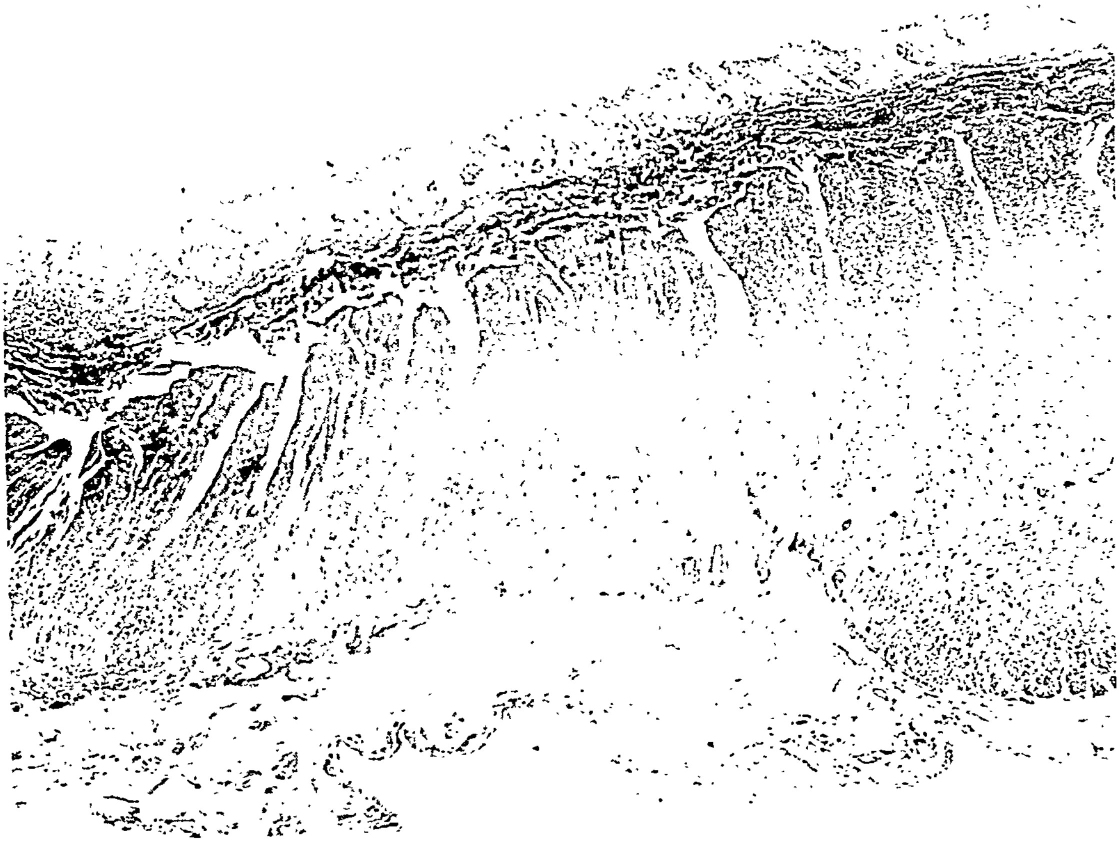

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Bevans

Scleroderma

PLATE 8

FIG. 10. Case 1. Mesenteric artery illustrating fibrous tissue proliferation, lymphocytic infiltration and edema of intima, and reduplicated internal elastica. Elastic tissue stain. $\times$ 1075.

FIG. 11. Case 2. Striated muscle showing disintegration of fibers. Hematoxylin and eosin stain. $\times$ 450.

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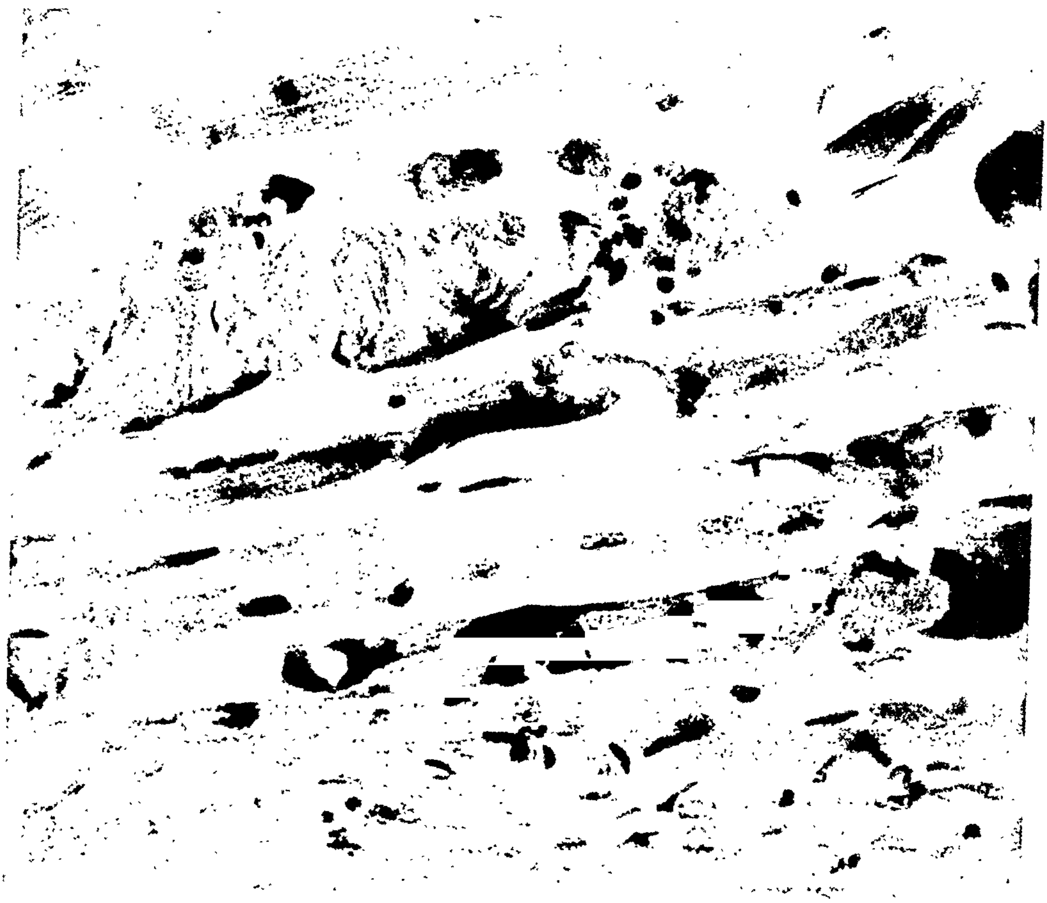

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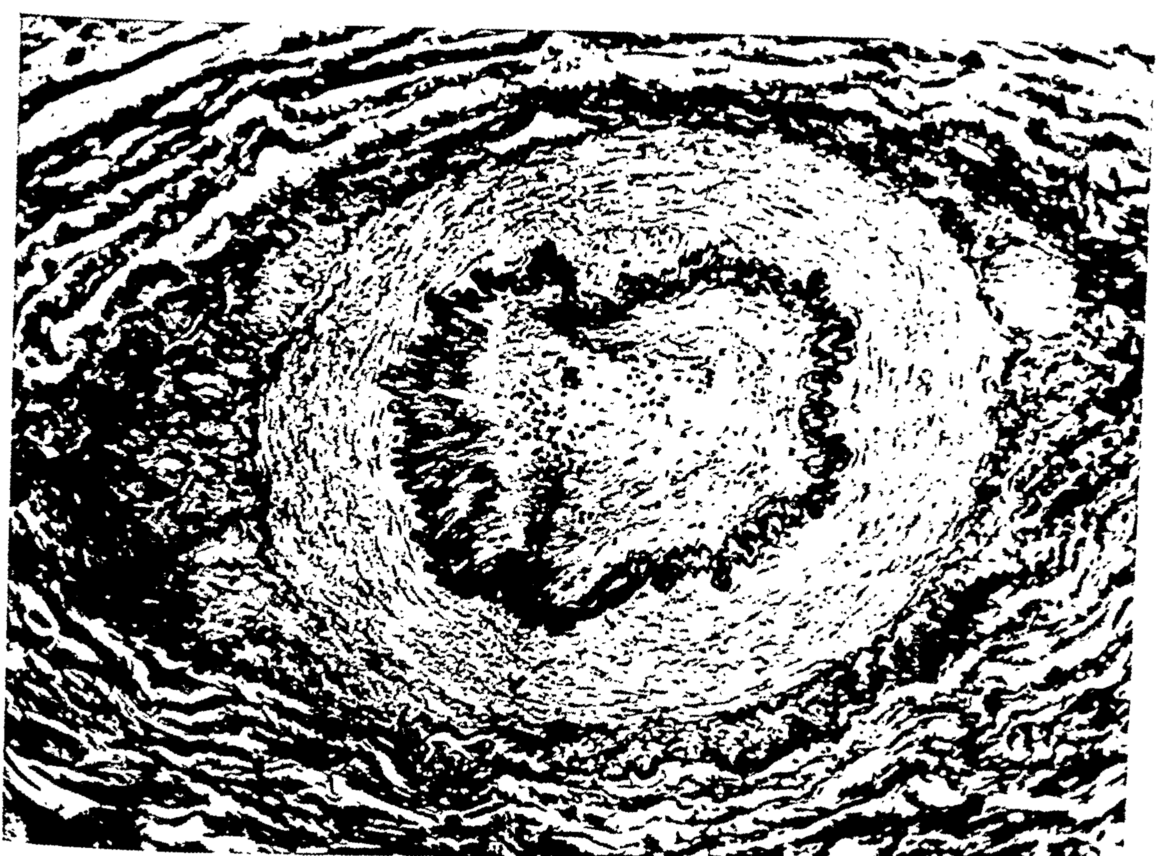

Plate 9

Fig. 12. Case 2. Small, thick-walled arteries and extensive replacement of alveoli of lung by fibrous tissue. In the few alveolar spaces which remain are desquamated cells. Hematoxylin and eosin stain. × 172.

Fig. 13. Case 2. Kidney showing partially necrotic glomerulus, pronounced arterial lesions, and a few casts. Hematoxylin and eosin stain. × 120.

Fig. 14. Case 2. Renal artery with necrosis of the muscularis, proliferation of the intima and penetration of the wall of the vessel by red blood cells. Hematoxylin and eosin stain. × 290.

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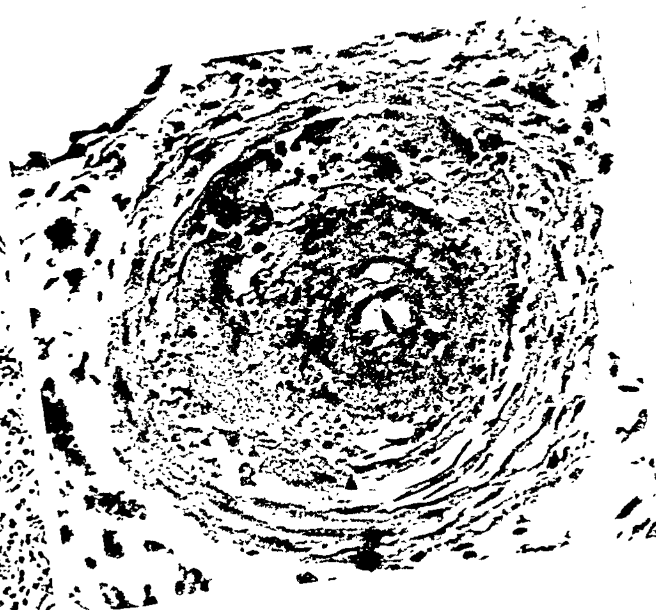

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Scleroderma

Bevans

HETEROLOGOUS MESODERMAL TUMORS OF THE UTERUS

Report of a Neoplasm Resembling a Granulosa Cell Tumor *

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Heterologous tumors of the uterus are not extremely rare. By far the most common neoplasms in this group are the mixed mesodermal tumors which occur in both the body and cervix of the organ.[1] At present, it is impossible to say with certainty whether the tumors occurring in the cervix differ fundamentally from those confined to the fundus. Pathologically, they are very similar.

Mixed mesodermal tumors are monodermal in origin and should be distinguished from the trigerminal teratomas, which are extremely rare. Morphologically, the former are sharply demarcated polypoid growths which occupy a submucosal position in the uterus and are composed of heterotopic malignant cells of various types. Embryonic myxomatous tissue is present in all of these tumors, and in many of them hyaline cartilage, striated muscle, bone and fat are to be seen. Many of the cells are well differentiated and the degree of malignancy is out of all proportion to the histological appearance. The prognosis is uniformly grave, most of the patients succumbing during the first year.

Many theories regarding the origin of these neoplasms have been proposed. The simplest explanation is that of neoplastic metaplasia.[2] However, it is extremely unlikely that adult tissues normally present in the uterus can be dedifferentiated into mesodermal derivatives which are normally not seen in that organ. Wilms [3] is of the opinion that these tumors arise from undifferentiated mesodermal cells which are displaced from the lumbar region during the descent of the Wolffian body. Lebowich and Ehrlich [4] have objected to this theory on the basis that no proved cases of this type of tumor have been reported as having developed along the course of the Wolffian ducts. In the light of our present knowledge, it appears most likely that these tumors originate from indifferent mesodermal cells which have retained their capacity for differentiation into mesodermal tissues of various types.[5]

Several case reports of tumors of this type are to be found in the recent literature, and excellent reviews of the subject have been given by Lebowich and Ehrlich,[4] Glass and Goldsmith [6] and Liebow and Tennant.[7] The purpose of this paper is to report the unusual case of a heterotopic mesodermal uterine neoplasm which possessed all of the morphological characteristics of a granulosa cell tumor. The case is of

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interest further in that it affords additional support for the contention of Fischel,[5] and others, that the granulosa cells are derived from the mesenchyme and not from the celomic epithelium as is ordinarily taught.

Report of Case

A white female, 44 years old, was admitted to the North Carolina Baptist Hospital on January 25, 1943, complaining of menorrhagia, a sense of pressure in the lower abdomen, and leukorrhea which was exaggerated immediately following menstruation.

She began menstruating at the age of 16 years and her periods occurred every 28 days with a duration of from 4 to 6 days. About 3 years before, she began to menstruate more frequently than formerly, and the interval between periods was reduced to 24 days. Concurrently, an increase in the duration and amount of flow was noted, menstruation now lasting from 6 to 8 days. She had been pregnant seven times and had had two miscarriages and five normal deliveries.

The physical examination revealed a fairly thin woman in no apparent distress. Her temperature was 37° C.; pulse, 87 per minute; respirations, 22 per minute; and blood pressure, 180 mm. of Hg systolic, 78 diastolic. The thyroid gland was normal in size and position. The breasts were atrophic and no masses were felt. On auscultation a soft apical systolic murmur was heard. Positive physical signs were not elicited from the lungs. The abdomen was soft and there were no masses or tenderness.

Pelvic examination revealed normal external genitalia and a parous outlet. A cystocele and rectocele were present. The uterus was normal in size and was freely movable; a third degree retroflexion was present. No masses were palpable in the adnexa. The cervix showed slight erosion around the external os but was otherwise not remarkable.

The hemoglobin was 76 per cent (Sahli) and the leukocyte count showed 5,200 cells per cmm. The studies on the urine were not revealing.

A vaginal hysterectomy and posterior repair were done under spinal anesthesia by Dr. G. C. Cook on January 27, 1943. The ovaries were normal in size and the left one contained a thin-walled cyst, 1 cm. in diameter, which was ruptured at the time of operation and was found to possess a smooth lining and to be filled with a clear fluid. The adnexa were otherwise normal.

The patient had an uneventful postoperative course and was discharged on the 17th hospital day. A follow-up examination 11 months after operation showed her to be free from her previous symptoms. No palpable masses were present in the pelvis at this time.

Pathological Examination

The specimen consisted of the entire uterus, which measured 11 by 6 by 5 cm. in its greatest dimensions. The cervix was large and boggy and the vaginal surface was smooth, except for an area immediately adjacent to the external os where irregular areas of reddish brown discoloration were seen. The external surface of the body and fundus was smooth and glistening. The endometrial cavity was found to be filled with blood. Attached to the fundus on its posterior surface was an oval-shaped, firm nodule which was covered by endometrium and measured 2 cm. in diameter. It was well encapsulated and occupied a submucosal position. The endometrium covering the tumor and im-

mediately adjacent to it measured 1 mm. in thickness, while elsewhere it varied from 2 to 3 mm. The myometrium averaged 2 cm. in thickness and was without evident gross lesions. On sectioning, the tumor was found to be yellow and moderately firm; heavy, grayish white bands radiated throughout its substance.

Serial sections of the entire neoplasm revealed in every instance a well circumscribed and encapsulated tumor which occupied the submucosal position (Fig. 1). The myometrium immediately adjacent to the tumor was atrophic but otherwise presented no abnormalities (A in Fig. 1). A careful search was made for extracapsular tumor cells but none could be found.

The endometrium which covered the tumor, and that immediately adjacent to it, was thin and atrophic, and the longitudinal axes of the glands were parallel to the surface (B in Fig. 1). Elsewhere, the endometrium was hyperplastic and composed of numerous irregular glands which were situated in active interstitial tissue. The glands were thickened and were frequently composed of several layers of cells. In most instances the tumor was sharply demarcated from the remaining endometrium, although at several points considerable intermingling of tumor cells and endometrial stroma was noted.

The tumor was composed of groups of epithelial cells which were separated from each other by irregular bands of connective tissue. These bands radiated throughout its substance and frequently showed hyalinization. In many instances the parenchyma and stroma were so arranged as to form diffuse sheets of epithelial cells and not infrequently they presented a cylindromatous pattern (Figs. 2 and 3). A definite tendency toward rosette formation was clearly demonstrated in many areas (Fig. 4). The epithelial cells possessed large, darkly staining nuclei with scant cytoplasm. These cells were morphologically identical with granulosa cells and were more or less uniform in appearance. An occasional mitotic figure was seen. Varying degrees of luteinization were present in different areas, and clusters of cells showing extensive vacuolization were scattered throughout the neoplasm (Fig. 5).

At several points a sharp line of demarcation could not be drawn between the tumor proper and the endometrium. At these points the tumor cells were intimately associated with the mesodermal cells making up the endometrial stroma. These mesodermal cells appeared to possess embryonic properties, and differentiation into both epithelial and spindle cells could be seen. In many instances within the endometrium proper there were formed clusters of rosettes which were com-

posed of lutein-like cells, some of which displayed vacuolization (Fig. 6).

Laidlaw's method for the demonstration of reticulum was employed. The epithelial cells were found to be devoid of fibrils, and the cylindromatous pattern previously noted was now even more pronounced (Fig. 7). Spindle-shaped cells could be seen among the argyrophilic fibers, which communicated freely with one another.

Frozen sections stained with scharlach R revealed large quantities of sudanophilic fat, which occupied an intracellular position. Additional material examined with the polariscope was not doubly refractive and was probably neutral fat.

Discussion

The most frequent heterologous tumor occurring in the uterus is the so-called mixed mesodermal tumor. It is probable that these tumors originate from pluripotential mesodermal cells which differentiate into one or more structures ordinarily originating from the mesoderm. There exists at present considerable confusion regarding the exact criteria to be satisfied before a tumor can be placed in this group. This is evidenced by the fact that, in independent reviews appearing in 1941, Glass and Goldsmith [6] accepted from the literature 94 tumors as mixed mesodermal neoplasms of the uterus, while Lebowich and Ehrlich [4] would admit only 12. The latter authors, following the example of Läwen,[8] insisted on the presence of striated muscle in the tumor for it to be acceptable. Since these tumors represent a distinct pathological entity and follow a rather typical course clinically, it would appear unwise to exclude the majority of them on rather arbitrary histological criteria.

In rare instances, heterotopic tumors appear in the uterus which are identical morphologically with neoplasms occurring characteristically in organs quite remote from that structure. Schiller [9] described a tumor occurring in the uterus of a woman, 47 years old, which was morphologically identical with the ordinary ovarian dysgerminoma.

The case we have reported represents the first recorded instance of a neoplasm originating within the uterus which was morphologically identical with a granulosa cell tumor. The possibility of such an occurrence has, however, been suggested by Schiller, and others. Extraovarian granulosa cell tumors are extremely rare. Ragins and Frankel [10] have described a large intraligamentous granulosa cell tumor which was removed from a Negress, 37 years old, who presented no evidence of tumor in her ovaries. Voigt [11] has reported a retroperitoneal granulosa cell tumor which occurred in the absence of neoplastic disease in

the ovaries. Walthard and v. Werdt,[12] Klaften[13] and Fauvet[14] have each described retroperitoneal recurrences following the removal of granulosa cell tumors from the ovary. Schiller[15] is of the opinion that these tumors arose independently of the ovarian neoplasms and had their origin in mesodermal rests which had remained retroperitoneal and had not come in contact with the ovary. Clinical evidence in favor of this concept is found in the complete cures which have followed the removal of recurrent neoplastic tissue. Such cures are not obtained in other recurrent ovarian carcinomata.

Most investigators are now of the opinion that the granulosa cells are derived from the mesenchyme and not from the germinal epithelium, as has been the orthodox teaching for many years. Histogenetically, our tumor is best explained by assuming an origin from mesodermal cells that had retained their potentiality for producing granulosa cells in adult life. In this case, as in others, the stimulus for tumor formation remains unknown.

Summary

Heterologous mesodermal tumors occurring in the uterus are not extremely rare. They are usually of the mixed variety and are best explained histogenetically by assuming an origin from pluripotential mesodermal cells which have remained dormant and for some unknown reason assume neoplastic properties.

Occasionally, mesodermal tumors are found in the uterus which are identical with neoplasms originating characteristically in other situations. Such a tumor is the dysgerminoma of the uterus reported by Schiller.[19]

In this paper the first case of a neoplasm originating in the uterus, which was morphologically identical with a granulosa cell tumor of the ovary, has been reported. It is presented as evidence against the view which is prevalent among many morphologists that the granulosa cells arise from the celomic epithelium.

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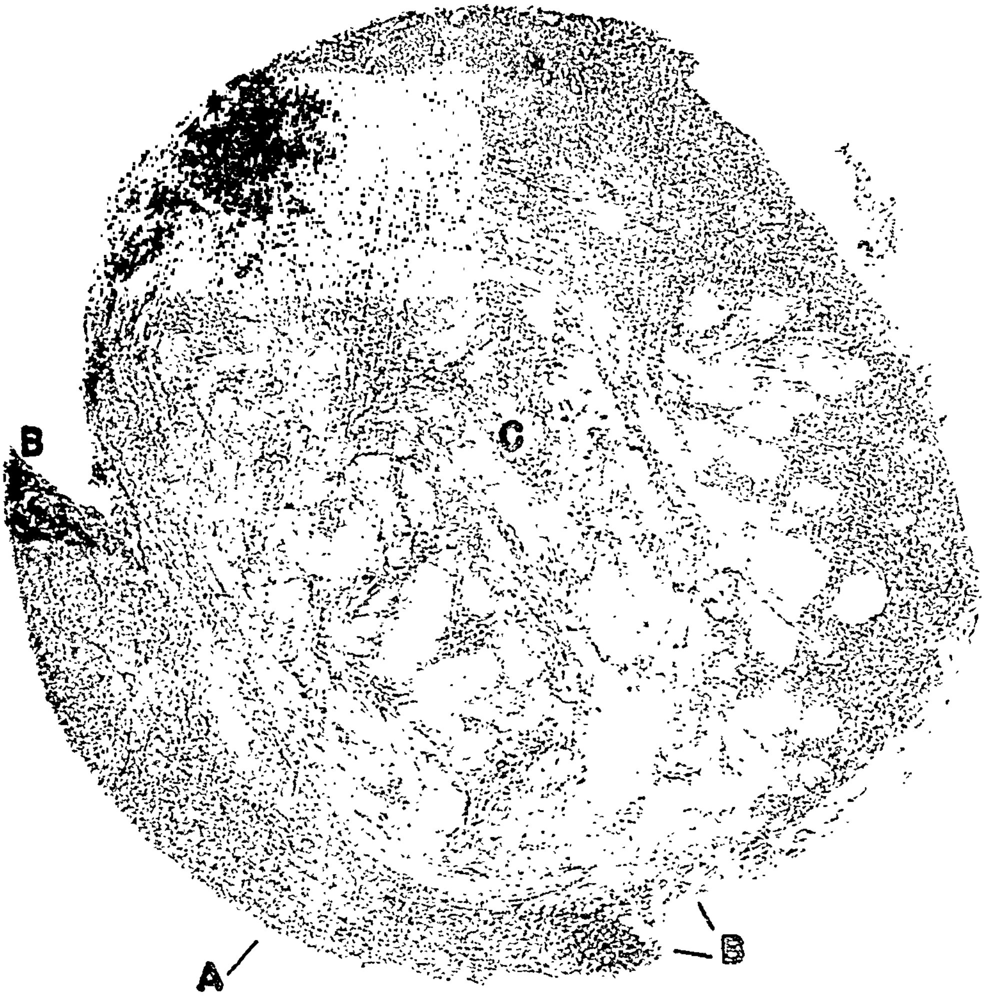
DESCRIPTION OF PLATES

PLATE 10

FIG. 1. A section through the entire tumor, which includes the adjacent myometrium. (A) Myometrium. (B) Endometrium. That portion which covers the tumor is thin and atrophic. (C) Tumor. × 7.

FIG. 2. Large groups of epithelial cells are seen and in certain areas there is a tendency towards a cylindromatous arrangement. × 90.

FIG. 3. A higher magnification of an area seen in Figure 2. × 320.

1


2

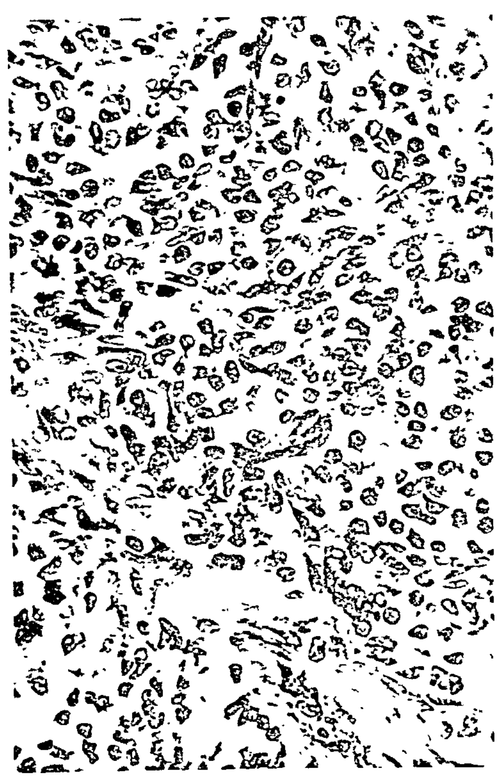


3

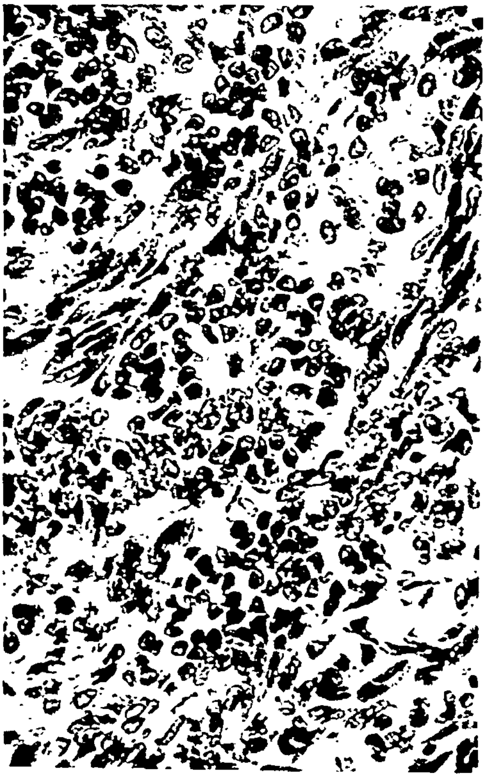
Plate 11

Fig. 4. A well formed rosette is seen in the center of the photograph. × 320.

Fig. 5. A group of epithelial cells, many of which contain neutral fat, are incompletely limited by dense fibrous tissue. Certain of the vacuolated cells show an alveolar grouping while others are scattered diffusely throughout the tumor. × 100.

Fig. 6. An area intimately associated with the endometrium. Mesenchymal cells here appear to be differentiating into both epithelial and spindle-shaped cells. The lutein-like cells are arranged in a characteristic grouping. × 90.

Fig. 7. Section stained for reticulum, showing the characteristic cylindromatous pattern and the absence of fibrils in the parenchyma. × 100.

4


5


6


7


Morehead and Bowman

Heterologous Tumors of the Uterus

ADENOMATOID TUMORS OF THE GENITAL TRACT *

Alfred Golden, Major, M.C., AUS, and James E. Ash, Colonel, M.C., USA

Fifteen cases of a tumor with definite anatomic features, limited to the epididymis, testicular tunics and the serosal surface of the uterine tube, have come to our attention in the past 2 years. A survey of the literature makes it probable that the same tumor has been seen and described by others under a variety of names (see "Discussion" below). Hinman and Gibson [1] (1924), Thompson [2] (1936) and Scalfi [3] (1936), in their reviews of the literature on tumors of the epididymis, spermatic cord and testicular tunics, agree that reported tumors of these sites are uncommon, and that those of the epididymis are exceedingly rare. However, in the past several years isolated case reports and reports of small series of cases have appeared in which the clinical and gross findings, the microscopic reports, and photomicrographs appear to be identical to those of the tumors to be described. Inasmuch as the reported series is still small, even in the aggregate, we believe it important to report our findings in this group of 15 cases, the largest series studied to date.

Clinical and Pathologic Aspects

In Table I there are summarized the salient features of the reported clinical findings and the topographic anatomy, and in Table II, the notable pathologic features of this tumor group.

From a consideration of the data contained in the tables the following condensation of the clinical details is possible: All but 2 of the tumors were present in males ranging from 20 to 68 years of age. Most patients were in the third and fourth decades of life. In only 1 of the 2 cases in females could the age be ascertained and in that instance it was 67 years. The tumor was discovered on routine physical examination, or as an incidental post-mortem finding or surgical finding, presumably without previous symptoms in 6 cases; and was associated with pain or tenderness, either with or without exertion, at the local site in 8 instances. Progressive increase in size was noted in 5 cases, usually on the basis of the patient's own testimony. A history of trauma was part of the clinical record in only 2 cases. In those instances where the tumor was discovered only incidentally, on either recorded physical examination or at operation for some other complaint, or at the autopsy table, there was, of course, no way of knowing how long it had been present; but when the patient did know of the tumor's presence, the shortest reported period prior to operation

* Received for publication, February 21, 1944.

TABLE I

Clinical Data on 15 Cases of Adenomatoid Tumors of the Genital Tract

A.M.M. Accession*	Sex	Age	Race	History, symptoms and signs	Location, topographic anatomy and source
97945	M	29	W	Discovered on routine physical examination. No symptoms noted.	Lower pole of the epididymis. Sharply demarcated nodule 1.5 cm. in diameter.
94094	M	23	W	Patient discovered a pea-sized, freely movable nodule in the region of the rt. testicle. There was pain locally on exertion. In 6 mos. there was a progressive increase in size up to 2 cm. in diameter.	Adherent to the tunica vaginalis, exact site not specified.
84615	M	28	W	History of blow to rt. testicle 10 yrs. before operation. Five yrs. later a small pea-sized swelling was noted which grew slowly in the last 1½ yrs. Another injury caused increase of mass to goose-egg-size with subsidence in 3 wks. Recent rapid growth to 2.5 cm.	In the "epididymis, independent of the testicle."
81346	M	32	W	Incidental finding in varicocele operation.	"Supernumerary body on the left testicle."
84648	M	26	W	Small growth in upper pole of left testicle with pain, of 3 yrs.' duration, with no increase in size. Onset attributed to blow to region.	Upper pole of testicle.
76268	M	44	W	Patient noted swelling, 10 yrs. prior to operation, in region of lt. testicle, with subsequent slight, gradual increase in size. Complaints of regional tenderness after long standing or walking. A pea-sized nodule present for 5 yrs. prior to operation.	Lower pole of epididymis.

TABLE I (Continued)

Clinical Data on 15 Cases of Adenomatoid Tumors of the Genital Tract

A.M.M. Accession*	Sex	Age	Race	History, symptoms and signs	Location, topographic anatomy and source
97904	M	21	W	Tumor of rt. testicle present for "some time." Tender to pressure and painful on sudden movement.	Lower pole of epididymis.
94348	M	26	W	Painful tumor in left side of scrotum of 8 mos.' duration, with threefold increase of size during that time.	Lower pole of epididymis. Shelled out easily at operation.
91983 A-36-1427†	F	67	W	Incidental autopsy finding.	Uterine tube.
S-13031‡	F	?	C	Incidental finding at operation.	"Encapsulated tumor on fallopian tube."
S-692‡	M	33	W	Duration and symptoms unknown.	Mass about the size of a bean was dissected away from the region of the rt. testicle.
S-15463‡	M	34	W?	Mass in region of testicle, known to be present for 3 yrs. prior to operation.	Tumor on the surface of the tunica vaginalis over the lower pole of the testis.
A-2633‡	M	68	C	Incidental autopsy finding.	Lower pole, lt. epididymis.
100648	M	22	C	Patient noted a small, tender nodule at the upper pole, lt. testicle, 2 wks. prior to operative removal. No history of trauma or infection.	Tumor nodule involving only the "capsule" of the upper pole of the lt. testicle.
101680	M	38	W	Nodule present in scrotum for 5 yrs., with very gradual increase in size during that time. Painful "at times."	Nodule in the globus minor of the epididymis.

* All cases except those noted below are from the Army Institute of Pathology, Army Medical Museum, and represent cases submitted through U. S. Army Medical Department sources. All cases have been incorporated in The General Tumor Registry, a subdivision of The American Registry of Pathology, The National Research Council, housed at the Army Medical Museum.

† Contributed by Dr. A. A. Nelson, Washington, D.C., from the Autopsy Series, University of Minnesota, School of Medicine, Department of Pathology.

‡ Contributed by The Division of Pathology, The National Institute of Health, Bethesda, Md.

Table II

Gross and Microscopical Observations on 15 Adenomatoid Tumors of the Genital Tract

A.M.M. Accession	Gross morphology	Microscopic morphology	Special stains	Notes
97945	Circumscribed, white firm tumor mass 1.5 cm. in diameter.	Mixed gland types. Mainly microfollicular, partly macrofollicular, and scanty, solid, cord-like formations. Fibrous stroma.	Fat stains (sudan IV in isopropyl alcohol) and mucicarmine stains negative in all cases for stainable material in the vacuoles or gland lumina. Best's carmine method for glycogen stained negatively in all cases, using formaldehyde-fixed tissue, or the paraffin blocks prepared from formaldehyde-fixed tissue as the *only* source material. Reticulum stains showed a well defined perifollicular meshwork in all cases. Phosphotungstic acid hematoxylin stains showed well defined cell borders without processes in the lining cells of the gland-like groups. The presence of smooth muscle bundles in some of the sections was confirmed by Masson's trichrome stain.	Serologic tests for syphilis, and routine blood counts were not contributory in any case.
94094	Dumbbell-shaped nodule 2.6 × 1.3 × 0.8 cm. in size. A thin capsule invested the whole. The cut surface was smooth, homogenous, translucent, and bulged slightly.	Mainly macrofollicular. Stromal background is dense, fibrous.		
84615	Encapsulated, globular, firm, elastic nodule, 2.5 cm. in diameter. The cut surface was grayish pink with irregular coarse areas of grayish white, and the whole had a whorled, fibrous appearance.	Mixed gland types: microfollicular, macrofollicular, and solid cord-like formations Small lymphocytic nodules present in the periphery. Fibrous stroma.		
81346	Portion of resected epididymis which overlay and partially enveloped a firm, gray, roughly spherical nodule within it, measuring 1.2 cm. in diameter. The mass appeared circumscribed and probably encapsulated.	Macrofollicular type predominantly. Diffuse, light, lymphocytic infiltration in a predominantly fibrous stroma.		
84648	Resected testicle, epididymis, and spermatic cord. An irregular, apparently circumscribed nodule was present, lying between the head of the epididymis and the base of the spermatic cord, without any evidence of invading regional tissues.	Mainly of the solid cord-like type, with occasional microfollicular formations. Stroma dense, fibrous.		
76268	The resected tail of the epididymis was found to be indurated. Cut section revealed a spherical, hard nodule 1.2 × 1.0 cm., which did not appear to be encapsulated. The cut surface was white and glassy, with faint yellow flecking.	Macrofollicular type predominantly. Stroma loose and fibrous.		No recurrence or metastasis 4½ yrs. after operation. Aschheim-Zondek test negative prior to operative removal.

TABLE II (Continued)

Gross and Microscopical Observations on 15 Adenomatoid Tumors of the Genital Tract

A.M.M. Accession	Gross morphology	Microscopic morphology	Special stains	Notes
97904	Poorly circumscribed nodule of firm, white, neoplastic tissue, measuring 1.0 cm. in greatest diameter, which encroached on regional testicular tissue without evidence of invasion.	Mainly microfollicular. Slight diffuse lymphocytic infiltration in the fibrous stroma, and an occasional small lymphocytic interstitial nodule.		
94348	Encapsulated, smooth gray-white nodule measuring 1.5 × 1.2 cm. Cut surface white, firm, tough.	Mainly microfollicular. Dense collagenous stroma. Large bundles of smooth muscle present in the periphery. Occasional interstitial lymphocytic nodule present.		
91983, A-36-1427	None available. Reconstruction from microscopic picture is that of a nodule extending from the serosa through the thickness of the tubal wall.	Mainly macrofollicular. Fibrous stroma.		
S-13031	"Encapsulated tumor in fallopian tube."	Mainly macrofollicular. Fibrous stroma.		
S-692	Mass about size of a bean dissected from region of "rt. testicle." (Microscopic section shows it to be epididymal.)	Microfollicular and solid cord-like types. Fibrous stroma.		
S-15463	Tumor nodule appearing as a part of the tunica vaginalis. The cut surface was "fibroid in character."	Mainly macrofollicular. Stroma partially fibrous with bundles of smooth muscle chiefly in the periphery. A few small clusters of lymphocytes and a slight diffuse spread of them are present interstitially.		
A-2633	Circumscribed, dense yellowish mass in the inferior pole of the epididymis, which is "walled off."	Mainly macrofollicular, and to a lesser degree microfollicular. Mainly fibrous background. Small number of smooth muscle bundles in the periphery.		
100648	Oval mass of pinkish gray tissue 0.5 cm. in diameter in "capsule" of testicle. Cut surface shows gray, faintly nodular, glistening, resilient tissue.	Mainly solid cord type. Partially encapsulated. Diffuse, slight, round-cell infiltration interstitially.		
101680	Globus minor of the epididymis received in which there was a firm, hard nodule, 1.0 cm. in diameter, which was partially encapsulated.	Mainly macrofollicular. Number of smooth muscle bundles chiefly in the periphery of the tumor. Numerous interstitial lymphocytic nodules. Moderate diffuse lymphocytic interstitial infiltrate.		

was 2 weeks and the longest 10 years. The size estimated by the surgeon or medical examiner was usually larger than the recorded gross dimensions as determined by the pathologist. The clinical appreciation of size probably took into consideration more than the tumor proper, possibly enlargement of the regional tissues consequent to blood and lymph stasis.

The following appears to be a reasonable composite gross description of this tumor group: The growths tend to be small, the largest in this series measuring 3 by 2 by 1 cm., the smallest, 0.5 cm. As a rule the tumor is globular, circumscribed and firm even to the point of induration. On section the cut surface varies from white to yellowish or pink and is glistening and may show either a smooth or finely fibrous stroma. (In one instance it was reported as being finely nodular.)

In our series the tumor was found either in the epididymis, the tunics of the testicle, or the serosal surface of the uterine tube. For tumors (2) from the last situation the gross descriptions are not available, but the microscopic sections show a neoplastic involvement, apparently growing into the underlying muscle coats from a superficial origin. In no instance was there any evidence, surgical or pathologic, of invasion of regional tissues or of metastases.

The microscopic anatomy of the tumors showed considerable variation. All of the tumors had a fibrous stroma varying from a loose collagenous meshwork to a dense, and in some instances, partially hyalinized fibrous stroma. Between the bundles of connective tissue, gland-like spaces were so distributed that fibrous stroma was present between all of them. Whatever the form of these glandular spaces their course lay in many directions, even in a single microscopic field. The glandular structures were of variable pattern, but the cell type was fairly characteristic. Some of the tumors tended to show a preponderance of one gland-like type over the other, but the rule was to find a variable picture, particularly in multiple sections. The variation in the gland-like structures was from almost solid cords (Fig. 2) of cuboidal and low-columnar cells to greatly dilated spaces lined by markedly flattened cells (Fig. 4). In Table II these are called "solid cord-like," "microfollicular" and "macrofollicular," respectively. In all of the tumors, examples of all types were seen. The majority of the cells contained vacuoles of variable size (Fig. 5), reaching exceedingly large proportions (Fig. 8), and producing a signet-ring appearance (Fig. 6). The vacuoles were always sharply demarcated. The nonvacuolated cells, best seen where the tumor formed cords, varied from cuboidal to low-columnar, had a finely granular, acidophilic cytoplasm, and a round or oval, centrally placed nucleus rich in chromatin. Cilia were

absent. No pigment was present in any of the cells. In many instances markedly vacuolated cells could be seen with only thin cytoplasmic strands connecting them (Fig. 6). In other places, gland-like spaces were lined by cells with shreds of cytoplasm still present along the free border (Fig. 7), suggesting gland formation by fusion of vacuoles. The gland lumina contained no material staining with hematoxylin and eosin, such as is seen in lymphangiomas. No blood cells of any kind were found within the lumina. This cell type is common to all of these tumors, as is also the pattern produced by these cells. This pattern is consequently considered the primary unit of structure for this tumor.

It was previously noted that the composition of the stroma was variable. Occasionally, bundles of smooth muscle were present in considerable quantity (Fig. 11). In none of these instances was the microscopic evidence convincing that the muscle was an integral part of the tumor. It appeared more reasonable to assume that the smooth muscle represented inclusion of pre-existing muscle in an expansile tumor growth.

An attempt was made to determine the nature of the vacuoles. Special stains for lipids were negative in all instances. The mucicarmine stain revealed neither mucinous granules nor mucin vacuoles in any of the sections. None of the material available for study had been preserved in ethyl alcohol. Therefore our attempts at staining these vacuoles with Best's carmine method for glycogen made use of formaldehyde-fixed wet tissue and/or the paraffin blocks prepared from formaldehyde-fixed tissue. The results were uniformly negative. The question, therefore, of the glycogen content of these vacuoles must remain open. The phosphotungstic acid hematoxylin stain revealed no fibrillar processes in any of the cells, even when they were present in solid cords, or when they lay in small clusters in the interstitial tissues. On the contrary, this stain revealed that the cell borders were well defined, smooth, and free of either brush borders or cilia. Reticulum stains showed a characteristic pattern of the reticulum meshwork (Fig. 10) rather intimately applied to the gland-like spaces in strands from one to three rows thick. There was no evidence of a basement membrane. The interstitial tissue contained a scattering of lymphocytes, with an occasionally admixed monocyte (Fig. 3). Plasma cells, eosinophils and neutrophils were absent. Occasionally, a cluster of lymphocytes produced a small nodule without a secondary center. Other portions of the same tumor, or other tumors, were without round cell infiltration. There was no evidence of the effects of chronic inflammation, such as scarring, adjacent to the lymphocytic foci. In none

of the sections studied was there any evidence of invasion of the regional tissues. Microscopically, the tumors could be shown to be either sharply circumscribed or encapsulated (Fig. 1). In most instances there was no marked compression of the regional tissues, suggesting that the rate of tumor growth was slow.

Discussion

Inasmuch as this tumor appears to be confined to the genital tract, origin from misplaced embryonic or fetal genital ridge is a tempting hypothesis. Accordingly, a number of testes and epididymides, with their adjacent tissues, from stillborn infants, and similar anatomic structures from fetuses, were subjected to frozen section study from slices made at close intervals. No structures resembling these tumors were encountered. There are no reports of cellular elements in the development of the embryonic genital ridge which resemble those of the tumor described above. It is conceivable that, since these tumors may arise from an abnormal group of genital ridge cells, the method of sampling employed is inadequate. One should examine a very large number of specimens before accepting or dropping such a postulate as to origin. The question of the tumor's genesis must remain open.

Differential Diagnosis

It is our impression that the tubular, acinar, follicular, or, at least, gland-like element of the tumor is composed of *epithelial* cells. The variation from low-columnar to flattened cuboidal cells growing with an epithelial cohesiveness is best illustrated in regions showing cord-like growth (Figs. 2 and 5). The tendency of these cells to develop vacuoles is another epithelial characteristic (Figs. 5 and 6).

Those authors who have considered the tumor endothelial in origin (Rigano-Irrera,[4] Charache,[5] Malisoff and Helpern,[6] Mercandier and Thomas[7]) appear to have accepted the flattened cuboidal appearance of the tumor where large spaces are formed as the cell type (Fig. 8). An examination of their photomicrographs and of our material shows an essential difference cytologically between these cells and endothelium elsewhere, even in angiomas. In the latter tumor group, lining endothelial cells preserve the typical endothelial, central nuclear bulge in a spindle-shaped cell which has sharply tapering ends. In our series of genital tract tumors the whole cell is flattened and never spindly.

Evans[8] proposed the reasonable hypothesis that these tumors are mesothelial in origin, because of their location on or near mesothelial surfaces either in the epididymis, tunics of the testicle or uterine serosa. Aside from the historic errors of oncologists in postulating mesothelial

origin to neoplasms of obscure origin, this hypothesis fails to explain the marked tendency towards vacuole formation and the gland-like special arrangement of cells. These properties are described neither for normal mesothelium nor for the more commonly accepted mesotheliomas, such as those of the pleura. Occasionally, one sees prominent mesothelium in various stages of inflammation, as in pleuritis, pericarditis and peritonitis. By using the comparison microscope it is clearly evident that the cells of this tumor are markedly different from even swollen mesothelial cells. In organizing or organized inflammations in such sites (pericardium, pleura or peritoneum) one may see pinched-off mesothelial cell clusters, still viable in a fibrous background. These, too, show marked cytologic differences when compared side by side with these tumor cells, and certainly show no tendency towards vacuolization. Finally, while it seemed obvious in Evans' series that there was an intimate serosal connection in his 4 cases,[8] no such clear-cut uniformity of continuity is present in our series of 15 cases.

The stromal background is variable, both in different areas of the same tumor and in different cases. The collagenous stroma may be of variable density. Smooth muscle may be present in isolated strands or in fairly heavy bundles. Our examinations convince us that both strands and bundles represent inclusions of neighboring muscular tissue. Therefore, a compound name such as mixed leiomyoma and lymphangioma (Malisoff and Helpern,[6] and Halpert [9]) does not appear justified. Similar objections may be raised against the designation "adenomyoma" (Sakaguchi [10] and Falconer [11])

In a discussion of pseudo-tumors of the epididymis, Mark [12] described a similar tumor, judging by the text descriptions and the accompanying photomicrograph. He postulated a chronic inflammatory lesion as the basis for the histologic picture. While it is true that a diffuse lymphocytic infiltration and even nodular lymphocytic accumulations are present in these tumors, there is no other evidence of inflammation; nor do any of the known chronic inflammatory conditions of these genital structures result in such tissue alterations.

Thompson [2] and Hinman and Gibson [1] reported similar, if not identical, tumors and described them as adenocarcinomas of a low grade of malignancy. In our series there is no evidence of invasion of the regional tissue even in our cases of 10 years' duration. Follow-up information is available in only 1 of our cases (A.M.M. Accession 76268) where in 4½ years following removal there was no evidence of either recurrence or spread.

On the basis of our analysis it appears to us that the primary unit of the tumor is epithelial in nature and that it tends to form gland-like

spaces and, therefore, deserves the name adenoma, as is suggested by Gordon-Taylor and Ommaney-Davis,[13] and by Blumer and Edwards.[14] However, the genesis of the tumor is obscure. We cannot be certain that these elements arise from a pre-existing glandular structure. In the present state of our knowledge it is proposed that the designation *adenomatoid* be given to this tumor type. The proposed name has the advantage of being morphologically correct and genetically neutral.

Natural History of This Tumor Group

The first impression one receives in examining these tumors microscopically is frequently that of disorderly epithelial proliferation. In our series a diagnosis of malignancy was frequently made by the primary examiner. Clinically and pathologically the evidence favors benignancy. Where long periods intervened between discovery of the tumor and operation, there was no histologic difference in the tumor type as compared to those removed soon after discovery. One does not expect this sort of constancy in a malignant tumor. When first found at autopsy, or incidentally at operation, the tumors are fairly large without evidence of local invasion or distant metastases. Mitotic figures are very rare in all cases. The question as to whether these tumors can become invasive and metastasize in time cannot be answered from the data available. Most of the tumors grew slowly, according to the clinical data. The clinical description of a period of rapid growth immediately antecedent to surgical removal does not appear to be borne out by microscopic examination of the tissue. There is neither evidence of mitotic cellular proliferation nor of marked regional tissue compression such as would be the case in rapidly expanding tumors. As has been suggested, some of the clinical impressions of increased size may be due to partial compression or obstruction of venous or lymphatic return from the regional tissues, producing an increase of size in the area involved. A history of trauma and subsequent growth was present in 2 cases. The relationship to trauma is obscure and there is no evidence in regional tissues that trauma may have played a rôle. One does not find areas of fat necrosis or organized hemorrhage, for example. Furthermore, it should be noted that identical tumors were found in 2 instances in this series in the uterine tube, again without microscopic evidence of trauma.

Biologic Activity of the Tumor Group

In 1 of our cases the Aschheim-Zondek test was performed prior to surgical removal and was reported as negative. In the case reported by Malisoff and Helpern[6] a positive test was recorded, but in that case discussion brought out that it may have been a false positive test.

SUMMARY

Fifteen cases of an apparently benign tumor of the genital tract in males and females are reported and the pertinent pathologic and clinical findings summarized. An argument has been presented for designating these neoplasms as *adenomatoid tumors*. *Adenomatoid tumors* of the genital tract have a well defined glandular pattern, as a rule, arranged spatially in many planes with considerable variation in size of lumina and cell structure. They may suggest malignant tumors on primary microscopic examination, particularly by the frozen section method. Both in our series and in reported cases there has been no evidence of malignancy if mitotic activity, local tissue invasion, and metastasis are considered. The clinical data also suggest the benign nature of the tumor group. The origin of this tumor is obscure.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

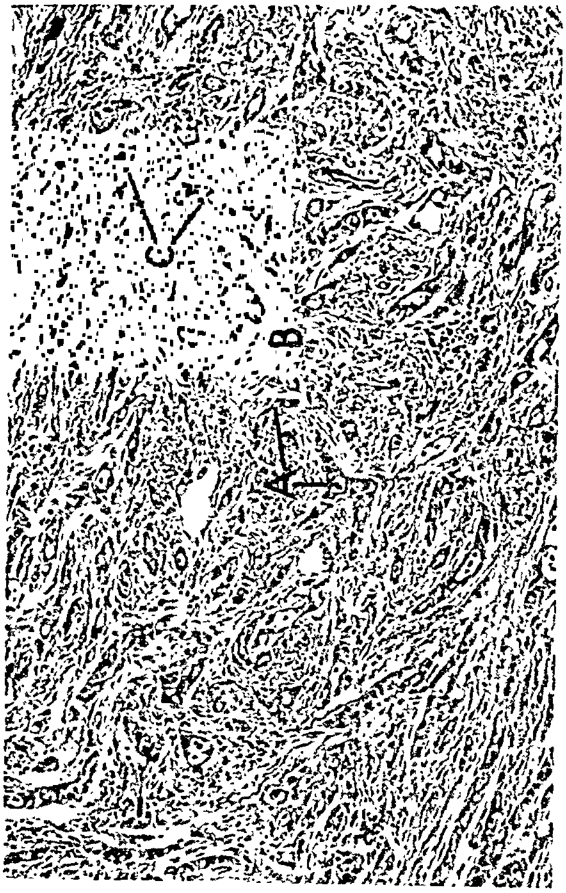
Plate 12

Fig. 1. Acc. 81346, Neg. no. 74712. Low-power view of the relationship of the tumor (A) to the epididymis (B). × 5.

Fig. 2. Acc. 84648, Neg. no. 74708. There is a predominantly solid growth in cords (A) in a dense fibrous stroma (B). Vacuolated cells are seen at C. × 118.

Fig. 3. Acc. 84648, Neg. no. 74709. The tumor pattern is microfollicular. Vacuolated cells are seen at A; scattered lymphocytes at B. × 118.

Fig. 4. Acc. 84615, Neg. no. 74714. The pattern is chiefly macrofollicular (A). Solid cords of cells (B) are present also. × 118.



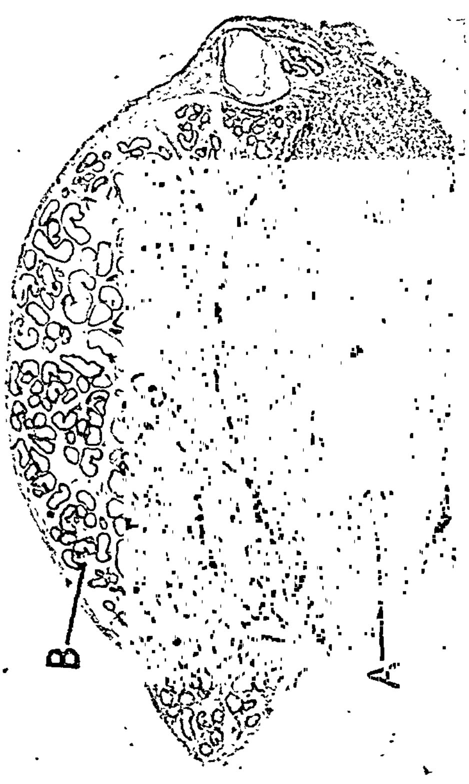


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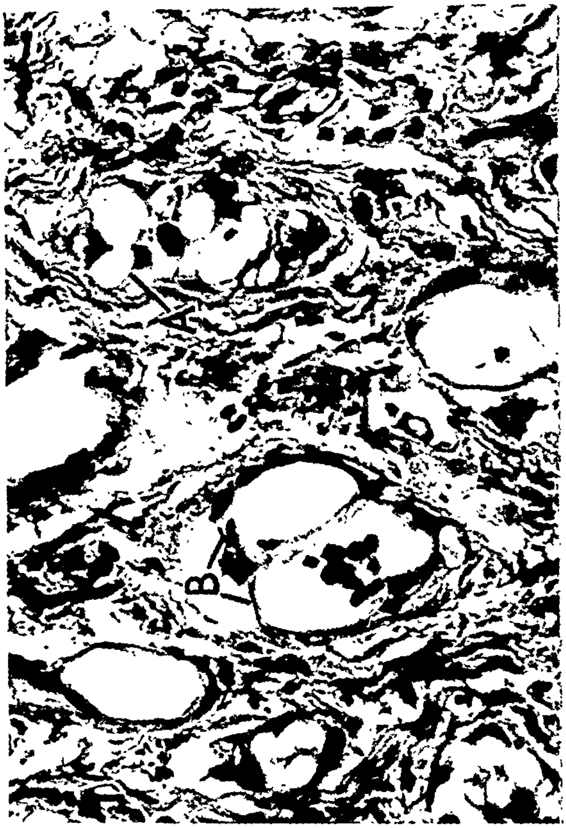
PLATE 13

FIG. 5. Acc. 84648, Neg. no. 74710. The vacuolated type of cell (A), of moderate size. × 395.

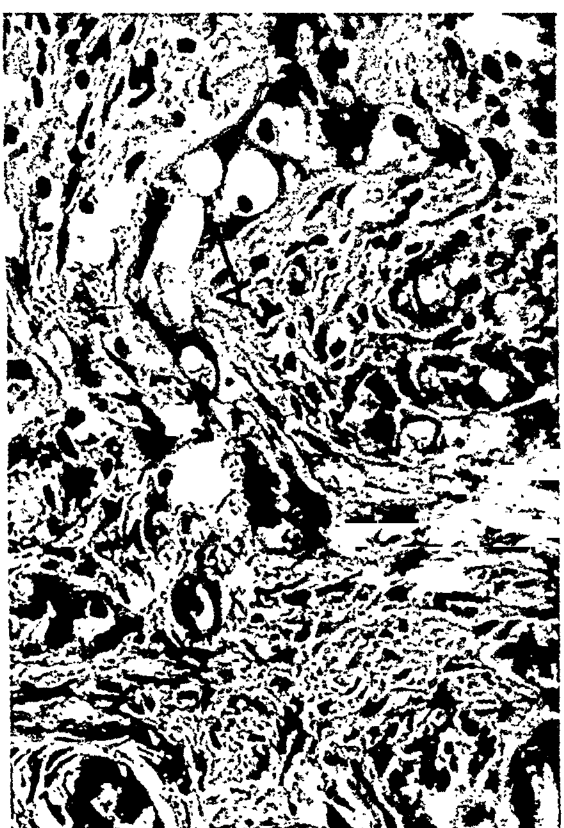
FIG. 6. Acc. 76268, Neg. no. 74705. Larger cell vacuoles producing signet rings (A), and very large, multinucleated vacuolated cells (B). × 395.

FIG. 7. Acc. 81346, Neg. no. 74711. A gland-like space (A) with cytoplasmic spurs along the free cell border. × 395.

FIG. 8. Acc. 84615, Neg. no. 74715. The cell type seen in the macrofollicular pattern. The whole cell is flattened, but is still cuboidal, and not spindly (A). There are vacuolated cell cords between the macrofollicular structures (B). × 395.


A
B


A
B


A

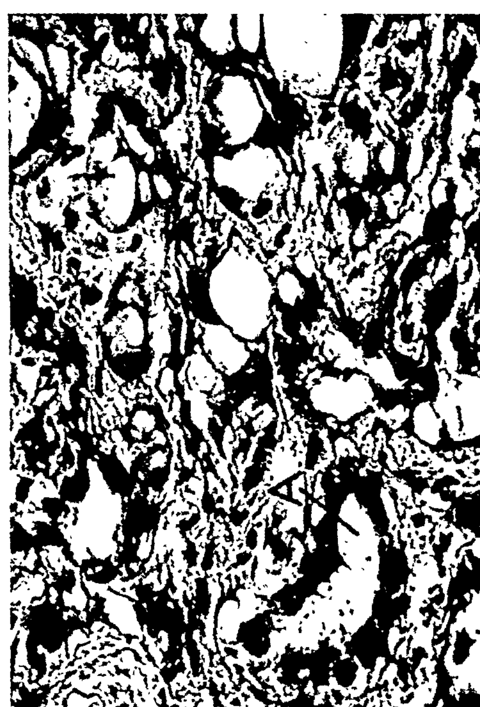
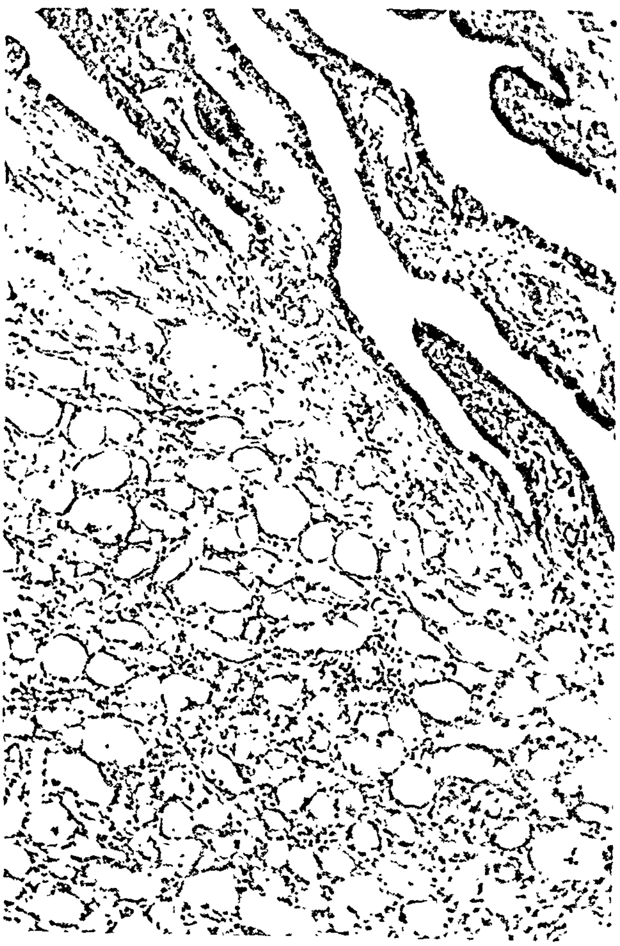

A

PLATE 14

FIG. 9. Acc. 91983, A-36-1427, Neg. no. 77134. The neoplasm present in one of the two cases of involvement of a uterine tube. × 74.

FIG. 10. Acc. 84615, Neg. no. 77131. The reticulum meshwork has a fine to coarse perifollicular arrangement. × 74.

FIG. 11. Acc. 84615, Neg. no. 77130. Smooth muscle bundle inclusions in this instance have an arrangement (A) which is almost myomatous. × 74.



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Adenomatoid Tumors of the Genital Tract

ACCESSORY SPLENIC TISSUE WITHIN THE SCROTUM

Report of a Case *

HARRY G. OLKEN, M.D.

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Accessory splenic tissue is no medical novelty. In fact, it is found so commonly in the course of routine autopsies that Lubarsch[1] stated that accessory spleens should be included in the normal anatomy of the adult. The reported incidence of accessory spleens at necropsy varies from 11 per cent[2] to 35 per cent.[3] The variation apparently depends upon the zeal of the particular investigator and the care with which his search is conducted.

The accessory splenic bodies vary markedly in size and may, at times, be quite numerous. Almost invariably they lie in close proximity to the spleen, being located in the hilum or in one of the surrounding ligaments. More rarely they are associated with one of the neighboring organs, such as pancreas or liver.[4]

Accessory splenic tissue within the scrotum, however, is a distinct oddity. Such an occurrence was first reported in 1913 by Sneath,[5] who described a splenic appendage attached to the upper pole of the left testis of a Negro, 45 years of age. This bulbous appendage was attached to the spleen by a narrow band which passed through the inguinal canal after traversing the peritoneal cavity and joining the spermatic cord. More recently (May, 1943) Emmett and Dreyfuss[6] described a somewhat similar case in which accessory splenic tissue was found within the scrotum of a white man, 47 years old. With their case report these authors included an excellent summary of the meager literature on the subject.

Report of Case

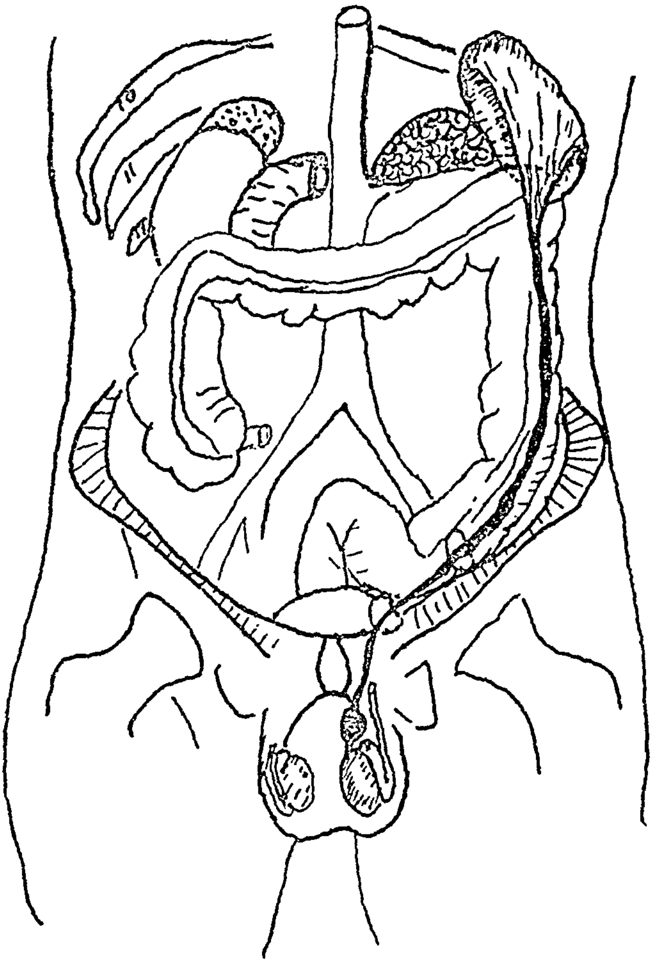
Clinical History. The patient, a white male, 56 years old, was admitted to the Lynn Hospital (case no. 110726) complaining of diffuse abdominal pain and hematemesis. A clear-cut history as to onset and progression of the symptoms was not obtainable as the patient was semicomatose and had been on an extended alcoholic bout. Physical examination revealed a moribund man, with a tender and spastic abdomen. The lungs were clear. A firm, nontender mass was felt within the scrotum, apparently attached to the upper pole of the testis. Temperature was 102° F., and the pulse rate was 140 per minute. On several occasions during the period of hospitalization the patient vomited a coffee-ground fluid.

In spite of therapy the patient died on the second hospital day, with a diagnosis of probable bleeding and ruptured peptic ulcer, or gastric carcinoma, with generalized peritonitis.

* Received for publication, February 15, 1944.

Autopsy Findings

At autopsy (Lynn Hospital autopsy no. A-43-52) an acute, bleeding, duodenal ulcer was found. The ulcer had perforated and had resulted in a generalized fibrinopurulent peritonitis. All of the thoracic and abdominal organs were normally formed with the exception of the spleen, and all showed the effects of the severe infection and associated toxemia.



Text-Fig. 1. Diagram showing the relationship of the spleen and its appendage to the abdominal viscera.

The spleen (weight, 200 gm.) was slightly enlarged. It occupied its regular position in the abdominal cavity but was misshapen. Arising from the anterior aspect was a tapering tail of splenic tissue which continued down along the left lateral wall of the abdomen as a firm cord. This cord was encased in a fibrous capsule and contained a core of brownish splenic tissue. It varied in diameter from 0.3 to 0.5 cm. At the left internal inguinal ring it joined the spermatic cord, passed through the inguinal canal and terminated in the form of a bulbous tumor-like mass measuring 1.8 by 1.0 by 1.0 cm. (Text-Fig. 1). This

lay close to the head of the epididymis and was attached to the tunica albuginea by a broad base (Fig. 1). As it was completely covered by tunica vaginalis, it was not visible until the sac was opened. The cut surface of this bulbous mass resembled normal splenic tissue, being composed of a reddish brown parenchyma surrounded by a dense fibrous capsule (Fig. 2).

A small accessory spleen, 0.8 cm. in diameter, was found within the abdomen close to the hilus of the spleen.

Microscopic Examination

Examination of the tissue mass which lay close to the head of the epididymis showed the typical histologic configuration of a spleen with a fibrous capsule, trabeculae, sinusoids and pulp (Fig. 3). There was a moderate inflammatory hyperplasia of the pulp, a reaction seen also in the abdominal spleen.

Discussion

In the first reported case of a scrotal spleen, Sneath [5] described, more truly, a splenic appendage which lay within the tunica vaginalis testis. In this respect it resembled the case herein reported. Since then other reports have described bands between the spleen and the genital organs, some of these bands consisting of typical splenic tissue.[7,8] That such bands are not always present, however, was shown by Emmett and Dreyfuss.[6] They reviewed 4 cases and added another of their own in which the scrotal spleen was in no way connected with the abdominal organ.

The appearance of this splenic tissue in so distant a place as the scrotum is readily explained on the basis of the close embryologic relationship which exists between the spleen and the urogenital organs. This has been well described and illustrated in previous reports.[5,6] Both the spleen and the testis make their appearance at about the fifth week of fetal life. The spleen appears as a thickening of the layers of splanchnic mesothelium of the dorsal mesentery of the stomach. The testis, arising as an indifferent sex gland from the medial surface of the Wolffian body, comes to lie in front of the primitive kidney on the posterior wall of the abdomen. The two organs are thus brought into close apposition, and fusion of the two is not inconceivable. Under such circumstances the testis, in its descent during the latter weeks of intrauterine life, might readily drag along a tail of developing splenic tissue.

Although accessory spleens usually arouse no great medical curiosity, they may offer an interesting clinical problem. They have been known to produce symptoms by torsion of the pedicle, by causing

STUDIES ON THE MOTOR CELLS OF THE SPINAL CORD
III. Position and Extent of Lesions in the Nuclear Pattern of Convalescent and Chronic Poliomyelitis Patients *

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Although the literature on the lesions of poliomyelitis is very extensive, it contains scarcely any data on the exact position and extent of lesions in the spinal cord. Probably one reason for this lack has been disagreement on the arrangement of motor nuclei in the cord; for in the absence of a dependable map of the nuclei one cannot very well say which are affected, and so it would be hardly profitable for anyone to follow a lesion through serial sections. Hence the character of the lesion has received more attention than its form and site.

Yet, if the nuclear pattern were known and so could be used as a frame of reference, it might prove worth while to study the disposition of the lesions. If any tendency to regularity were found in the pathological picture, this would obviously be of significance, enabling us to visualize the process of invasion and recovery and furnishing us with evidence as to the route followed by the virus into the spinal cord.

In two previous communications[1,2] I have described and confirmed a map of motor-cell distribution in normal human spinal cords; and thus it is now possible to refer lesions to an established pattern, and to state exactly what parts of the pattern have been affected.

Accordingly a number of cords from convalescent and chronic cases of poliomyelitis have been studied with respect to the disposition of the lesions, and are reported on in this paper.

Literature

As remarked at the outset, numerous authors have discussed cellular pathology in poliomyelitis; and they have paid particular attention to the motor cells of the ventral horns, since these are the classical site of lesions. Warburg,[3] in particular, has described the appearance of cells in chronic cases, providing a good bibliography and summary of preceding papers on this topic. But of these, Schwalbe[4] alone has paid any attention to the positions and extent of the lesions; he found that they started centrally in the ventral horns and that marginal cells were

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most likely to survive. Since then, Horányi-Hechst [5] has made a similar report; from a material of 38 cases he found that in the cervical segments the central groups suffer most severely, the dorsolateral less, and ventrolateral still less; while in the lumbosacral region the medial groups suffer more severely than do the lateral. He also found that the 2nd and 3rd lumbar, and 6th, 7th and 8th cervical segments were most frequently and extensively involved, and that, whereas the most rostral and caudal portions of the thoracic region were frequently involved, the intermediate region was rarely affected. Hurst [6] mentioned that an infiltrative process lay immediately adjacent to the central canal in one case, and Peers [7] commented on survival of caudal groups, but even this degree of localization is lacking in most accounts of the lesions.

Material and Methods

The following account concerns cords from 5 human subjects who had suffered from poliomyelitis and subsequently died from other causes. The lapse between onset of the poliomyelitis and death was at least 2 months, and in 1 case was 50 years. To these was added the cord from 1 monkey which had convalesced about 3 months after a severe attack. The human cords, as may be seen from the acknowledgments, were obtained from widely scattered sources and under a variety of circumstances. They had an age-spread of 7 to 61 years, and were all from male subjects.

Complete serial sections were cut from material embedded by the routine paraffin method and mounted *in toto,* excepting every eleventh section which was mounted with its fellows separately in case it were found advisable to study any level with an additional stain. The main series were stained with toluidine blue, and some of the auxiliary series with hematoxylin and eosin.

The sections were studied with a vertical projector by which charts were made after the method described in previous papers of this series, resulting in enlarged tracings each showing cumulative cell distribution in a series of sections. Lesions were determined by defects in the recognized cell pattern at any level, and by asymmetry between the two sides. These charts were made of the nuclear masses supplying the limbs, at short intervals where the pattern seemed normal, but for all sections where lesions arose.

Observations

The individual reports gain in interest and significance by summarizing them in advance of their presentation.

A very definite and consistent trend was found in the localization

of the lesions. The dorsal and medial nuclei were the most frequently attacked and often suffered alone, whereas the lateral and ventral nuclei were less frequently attacked and then only when the dorsal and medial nuclei were also destroyed. When a nucleus was destroyed only in part it was always the dorsal or medial portion that suffered. In other words, the invasion appeared always to radiate from a dorsomedial point.

All degrees of invasion were observed, from those in which there was only a small defect in the most medial or dorsal nuclei (case 70) to those in which all motor nuclei through a whole limb area were destroyed excepting a few cells around the rim of the ventral horn (case 73). Each example added something to the consistent picture of a process that had started dorsomedially and eaten into the lateral cell mass, each showing the process arrested at a different stage. Every lesion, great or small, was roughly fusiform, thrusting most deeply into the nuclear mass at an intermediate level, and less deeply at levels rostral and caudal to this, until it tapered to nothing; this gave the impression that each began from a single point and spread longitudinally as well as transversely.

A widely penetrating lesion did not necessarily have a great caudorostral extent but might be only 1 mm. long (case 108); while a lesion that encroached very little on the nuclear mass might extend through entire segments (case 70); but in most cases the degree of transverse and longitudinal extent was more nearly proportional. Again, a region otherwise denuded of cells might show an almost complete nuclear pattern in a few isolated sections (case 86).

In regard to the levels at which lesions appear there is no regularity apparent in this small series of cases.

The following detailed reports on the individual cords are arranged in order from that of the case showing the least involvement to that showing the most. Each description begins caudally and passes rostrally. Throughout, the term "cell" should be understood as referring to motor cells of the ventral horns only. For nuclear numbers, see Text-Figure 1.

Case 70

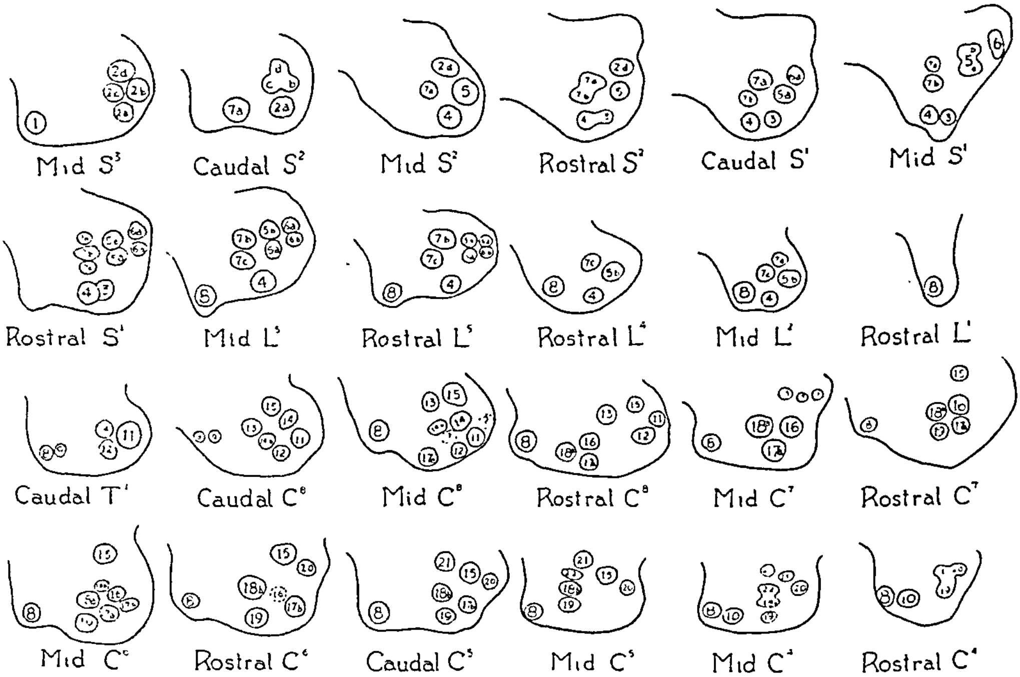
Male, age 61. Cause of death: exsanguination from penetrating gastric ulcer. Poliomyelitis "in childhood." Muscular findings: right psoas definitely smaller than left, muscles of right thigh slightly atrophied anteriorly and medially.

In the lumbosacral region the nuclear pattern was complete and marked; but on the right, nucleus no. 5 and, to a lesser degree, no. 6, were somewhat more sparse from about mid-S1 to about mid-L5. In the cervical region the pattern was poorly marked; at two levels there was a medial defect, slight but quite definite. The exact nuclei involved could not be determined because of the indistinct pattern (Text-Fig. 2).

Involvement of nuclei nos. 5 and 6 confirms the general thesis, especially as these nuclei are very far dorsal in this specimen. That no. 5 should suffer most severely is also typical. Localization of psoas and perhaps quadriceps and abductors in nos. 5 and 6 is a contribution to nuclear identification. The cervical lesions are typical.

Case 108

Male, 30 years of age. Cause of death: subacute bacterial endocarditis. Poliomyelitis at age of 2 years. Muscular findings: equinus deformity of right foot and general muscular atrophy of leg and thigh.



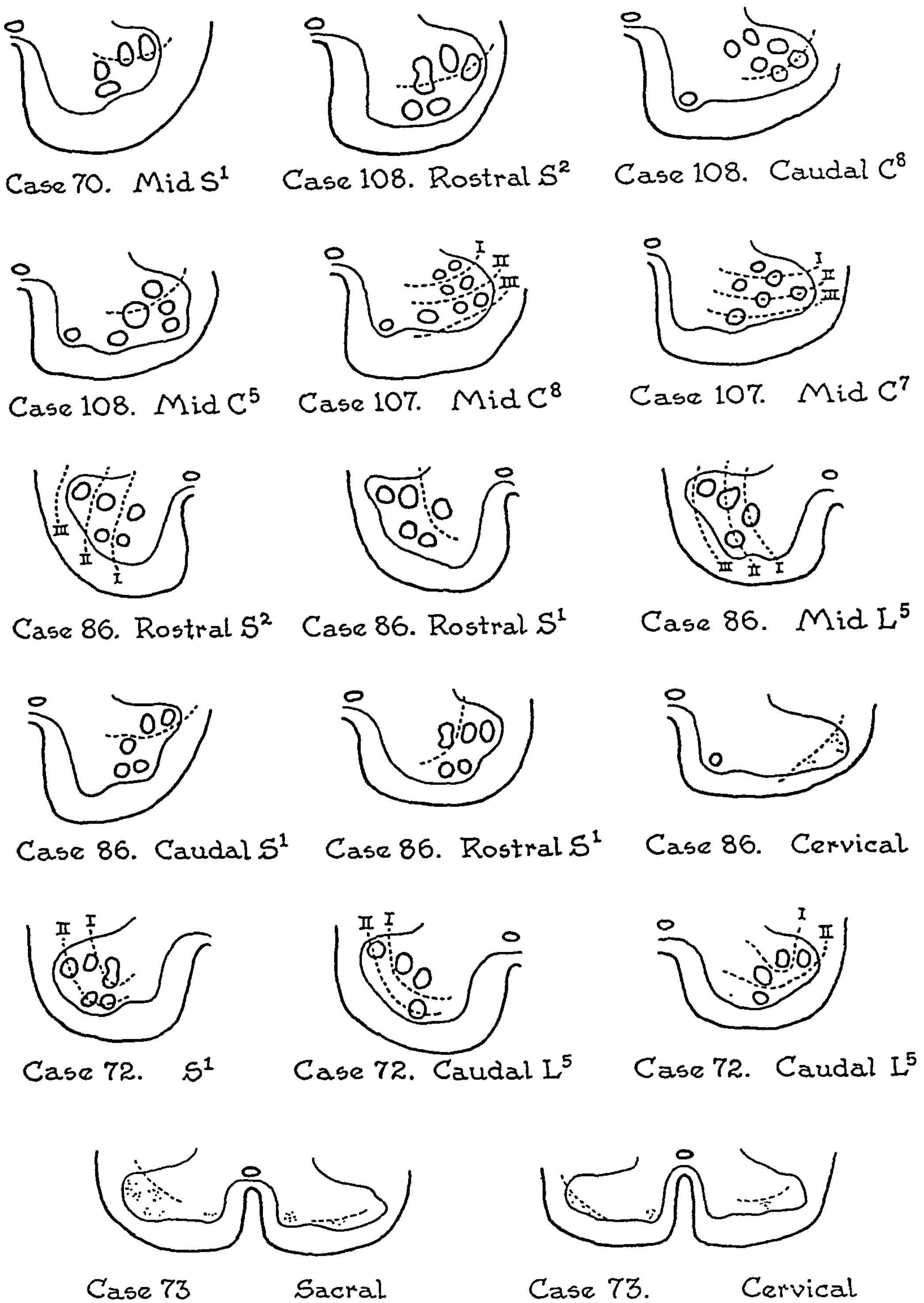
Text-Fig. 1. Diagrams of the ventral cell column cut in transverse section at the levels indicated. (Reproduced by permission from *The American Journal of Anatomy,* 1943, **72,** 29–38.)

In the lumbosacral region: on the left, all nuclei were normal; on the right, nos. 3 and 4 were normal, nos. 7 and 6 were present but sparse, no. 5 was lacking excepting scattered groups in the rostral segments, no. 2 was entirely lacking. In the cervical region: on the left, all nuclei were normal; on the right, in caudal C8, there was an almost complete lack of cells for about 1 mm., surviving cells lying at the lateral and dorsal margins of the horn, and at about mid-C5 medial groups, probably nos. 18 and 19, became sparse though still represented.

These lesions all support the general thesis, except that it is unusual for no. 7 to be spared at all when no. 5 is destroyed. The leg lesions conform very well to the paralytic picture and so make a further contribution to nuclear identification; it has been suspected that nuclei nos. 3 and 4 have to do with the glutei, which are presumably intact in this case, while no. 6 has to do with the iliopsoas, also presumably intact. Survival of some lower leg muscles should account for the equinus and may correspond to the survival of cells in no. 7.

Case 107

Male monkey, age unknown. Cause of death: intentional exsanguination followed by perfusion with a 4 per cent formaldehyde solution. Infection occurred 2 months



Text-Fig. 2. Cross sections through the lesions as described in the text.

Dotted lines represent the extents of lesions; dotted lines designated by Roman numerals represent successive encroachments of a lesion at successive levels. A dotted line passing through a nucleus signifies either that that part of the nucleus is destroyed or that the nucleus as a whole is thinned out.

In the examples from cases 86 and 73 in which outlines of nuclei are not shown, the level, and consequently the nuclear pattern, could not be determined. The dots represent the cells found through a considerable thickness of material.

A few lesions have been omitted because of obvious difficulties in representing them diagrammatically, or because they are duplicated by examples already shown.

and 23 days before death. Muscular findings: lower limbs apparently totally paralyzed; tail, perineum, upper limbs normal and slightly hypertrophied.

In the lumbosacral region all nuclei were destroyed except no. 1 and small caudal portions of nos. 2 and 4. In the cervical region: on the right, all nuclei were normal; on the left, at about mid-C8, all nuclei vanished for a few sections only, beginning with the most medial and ending with the most lateral, and reappearing in the reverse order; and again, about mid-C7, all the nuclei vanished beginning with the mediodorsal, and up to caudal C6 only a few scattered cells could be seen along the ventral boundary, after which all nuclei reappeared abruptly.

All of these lesions confirm the main thesis. The cervical lesions are particularly striking in this case, in view of the hypertrophied arms.

Case 86

Male, age 7, colored. Cause of death: apparently respiratory complications due to poliomyelitis. Infection occurred 2 months and 5 days before death. Muscular findings: stiff neck, paralysis of right arm, weakness of left, apparent involvement of respiratory muscles.

In the lumbosacral region: on the left, no. 2 began weakly and vanished about caudal S2; about rostral S2 nos. 7 and 4 vanished abruptly, followed at brief intervals by nos. 3, 5 and 6—*i.e.*, all nuclei present at that level—and they reappeared in the same order; in rostral S1 no. 7 again vanished for a short distance; finally, in mid-L5 the whole mass faded out in the same order as before—7, 5, 4, 3, 6—only no. 6 reappearing and continuing to the end of the enlargement. On the right, in caudal S1, nos. 5 and 6 faded out, reappearing about 1 mm. more rostrally; in rostral S1 no. 7 faded out briefly; and in caudal L5 the whole mass faded out in the same order as the two left-side examples, and did not reappear. In the cervical region few cells survived; a small group appeared at two levels—in the absence of a nuclear pattern it cannot be said which levels—at the extreme lateral tip of the left horn.

All seven lesions in the lumbosacral region support the general thesis. The surviving groups of cells, rostrally in the left lumbar region, and in the cervical region, offer further support.

Case 72

Male, 54 years old. Cause of death was undetermined; sudden respiratory seizure. Poliomyelitis at 15 years. Muscular findings: severe paralysis of the left leg. Only the lumbar enlargement was available.

On the left side: in caudal S1 nucleus no. 7 faded out, followed in order by no. 5 and parts of nos. 3, 4 and 6, the latter being represented by marginal cells; about rostral S1 the nuclei reappeared in the same order; in caudal L5 no. 5 disappeared, followed by no. 7, and in rostral L5 nos. 4 and 6 also disappeared, all but a few cells on the margin of the horn persisting for 2 or 3 mm. On the right side: in caudal L5 no. 5 disappeared followed by nos. 7 and 6; in rostral L5 no. 6 reappeared transiently; in caudal L4 all cells vanished except a few on the ventral margin of the horn which persisted for 2 or 3 mm.

Three large lesions and the positions of surviving cells all support the general thesis.

Case 73

Male, age 52. Cause of death: sarcoma. Poliomyelitis at 22 years. Muscular findings: paralysis of right arm, leg and erector spinae. Portions of the lumbosacral and cervical regions were available.

In the lumbosacral region: on the right, all nuclei but no. 1 were lacking, though a few cells were found at the ventral margin of the horn; on the left side there

seemed to be a general deficiency of cells, but this could not be confirmed since there were no right-hand nuclei for comparison, and no single nucleus was lacking at any level. In the cervical region: on the right, all nuclei were lacking but no. 8, but a few cells survived at the ventral margin; on the left, more cells had survived, concentrated ventrally, but the pattern was so disrupted that no nuclei could be identified.

The surviving cells confirm the thesis. It is interesting to observe the scattering of cells that have survived, presumably for many years, in an almost entirely denuded and atrophied region. And it is amazing that a person should have survived with motor cells so depleted.

Discussion

The regularity in position of the lesions is as complete as it was unexpected. In all 6 cases, comprising nearly a score of distinct lesions, there is no exception to the rule that cell destruction appears to start dorsomedially and to spread ventrolaterally. To this finding must be added the testimony of the two previous investigators, Schwalbe [4] and Horányi-Hechst,[5] who localized lesions in the cord, testimony which is in exact agreement with the present findings. Horányi-Hechst, in particular, with his 38 cases, adds weight to the conclusions; but his localizations are somewhat rough and generalized and he does not make clear that the ventral and lateral lesions are always accompanied by dorsal and medial lesions, but merely indicates a greater frequency of the latter type; nor does he describe the extent of the lesions. He worked entirely on acute or early convalescent material, which is much more difficult to analyze than is chronic, and had, of course, no reliable or easily available map of the nuclei. Nevertheless, there can be little doubt as to his meanings, and the outstanding thoroughness of his paper in other respects indicates that his findings are perfectly reliable. As to his statistics on the segments most frequently involved, comment will be more appropriate after my larger series of cords with acute lesions has been studied, but in the present series my findings agree with his.

At first sight the uniformity of the findings seems out of keeping with the clinical capriciousness of the disease. Of course, within the limitations hereby introduced there is still room for considerable variation: besides variation in the extent of damage and the number of lesions from subject to subject, the level of the lesions is apparently not restricted, even if certain levels are preferred; a lesion may involve one dorsomedial nucleus in one case and another in another; and its spread may be more ventral or more lateral. At the same time it must be remembered that certain muscles are traditionally susceptible to poliomyelitis, such as the tibialis anticus, opponens pollicis, and deltoid. It has, of course, been argued that the susceptible muscles

are only apparently so, for their paralysis is very obvious and so would be more easily and frequently detected, and this may be true to some extent. But the present findings very definitely indicate that some nuclear groups, and consequently some muscles, are really more apt to be affected than are others. Hence the findings are certainly not in disagreement with the clinical picture of the disease.

This means that an observer has at least a helpful indication in distinguishing muscles paralyzed by destruction of their motor neurons, and thus irretrievable, from those paralyzed or weakened by disuse, spasm, stretching, or other secondary influences, and thus possibly retrievable. For it could be inferred that if certain muscles were spared, those whose nuclei lay more ventrally or laterally were probably also spared, appearances to the contrary; while conversely, if certain muscles are definitely affected, those whose nuclei lay more dorsally or medially would almost certainly be affected also. Likewise, muscles whose nuclei lay between evident zones of total destruction and successful resistance could be watched and treated with appropriate care. In brief, the clinician can build up a clear picture of what is happening in the cord.

To attain these advantages it would, of course, be necessary to determine which nucleus corresponds with each muscle. So far this has been accomplished only fragmentarily, as will be discussed below.

The fact that in many cases a lesion appears to radiate from a single focus is very striking. It indicates that the virus may be transmitted to the cord by one, or a small group, of fibers. More widespread lesions can be accounted for either by more prolonged spread from one focus, or by a greater number of foci. In any case the number need not be very great, for a dozen lesions would quickly overlap to obliterate all motor cells in a limb region. Groups of cells surviving in an otherwise denuded region can be interpreted as lying at the juncture of two focal fields of invasion.

Nucleus-Muscle Relationships

Some evidence is presented on nucleus-muscle relationships, and the foregoing discussion illustrates the need of information in this field. Hence a brief survey of present knowledge of such relationships may not be out of place. Destruction of nerves and study of resultant chromolysis is rarely successful; a number of studies based on this method, notably those of Marinesco [8] and De Neef,[9] have produced contradictory results which, moreover, cannot be reconciled with my map of the motor nuclei; personal experiments of this type have met with little success. Long-standing or recent amputations seldom—

never in the few cases I have seen—show a recognizable reaction. Romanes'[10] study on a specimen with a congenitally deficient limb, and similar work reviewed by him, provide definite but rather generalized information. Cords from patients with poliomyelitis whose muscular picture has been carefully studied are rare, as shown by the extensive survey required to obtain those here discussed.

Frôm the sources referred to it can be accepted confidently that nucleus no. 1 supplies perineal muscles; no. 2, intrinsic muscles of the foot; no. 8, the spinal musculature; and nos. 11 to 15, the hand. Probably nos. 3 and 4 supply the glutei; no. 5, the quadriceps; and no. 6, the iliopsoas; while no. 15 has shown reaction to lesions of the ulnar nerve. Obviously, these localizations can at best be only partly correct, for they leave out many important muscles, including almost the whole arm.

The present findings indicate that no. 6 does supply the iliopsoas, and no. 5 the thigh muscles, and add that no. 7 supplies some lower leg muscles. Confirmatory evidence is offered by the fact that nos. 3 and 4 are frequently spared as are the glutei, while no. 7 is frequently involved as is the tibialis anticus.

The present study illustrates the danger of drawing conclusions from sections taken at only one or two levels in a region. Such sections might well pass through levels that were completely atypical for the region as a whole, showing many cells when the bulk of the region was almost denuded, as in case 86 cervical, or the reverse, as in case 107 mid-C8. If a cord is to be analyzed carefully, numerous or, preferably, serial sections should be taken.

Finally, the occurrence of limited but severe lesions in regions where the muscular picture would not lead one to suspect them is interesting. This was particularly so in the case of the monkey (107), where careful dissection showed the arms not only normal but hypertrophied. Such a condition leads one to suspect that many cases of poliomyelitis occur with involvement of motor cells of the spinal cord and yet without overt paralysis. This agrees with the findings of Howe and Bodian[11] (1942) and furthermore suggests that yet more extensive lesions might occur with no more than a muscular weakness which would not be classed as paralysis. One not infrequently observes supposedly normal persons with awkwardness of gait, posture, or gesture which might well result from such a lesion; and this accords with the prevalence of immune substances in the blood of many supposedly normal adults. This would at least reduce the number of cases occurring with, supposedly, no neural involvement.

The reason for the preferential destruction of dorsomedial nuclei is a matter of vital importance, since it bears directly on the natural history of the disease. But one can hardly theorize on the basis of six subjects. I feel that a greater insight into the question will be obtained when I have completed a large series of cords from acute cases, now in course of preparation.

Summary

The motor nuclei of the limb region were studied in cords from 5 human subjects and 1 monkey convalescent from, or with residual lesions of, poliomyelitis.

All lesions involved the dorsal and medial nuclei first and most severely.

Lesions in general were not diffuse but each appeared to arise from a single focus.

Knowledge of this trend may help the clinician in visualizing what is going on in the cord of a patient; but more satisfactory data on nucleus-muscle relations are needed for this purpose.

Evidence was found as to the functions of certain nuclei.

The findings illustrate the hazard of studying cases of poliomyelitis from single sections, which may give an entirely unrepresentative picture.

Motor cell destruction is found without recognizable paralysis, suggesting nervous involvement in many so-called abortive cases.

The force and conclusiveness of these findings would be greatly increased if the number of cases could be enlarged. If any reader can supply a cord from a convalescent or chronic case of poliomyelitis it would be most gratefully received.

I wish to express my warm appreciation for the very generous cooperation received from many quarters in pursuance of this work. State and Provincial departments of health, departments of pathology, hospital staffs, private doctors, and scientific journals have all aided in canvassing the continent for material. For the specimens used in the present paper I am indebted to: Dr. I. Erb, Sick Childrens' Hospital, Toronto; Dr. E. Linell, Department of Neuropathology, University of Toronto; Dr. E. B. Krumbhaar, University of Pennsylvania; Dr. W. Mathews, Shreveport Charity Hospital, La.; and Dr. R. H. Gourlay, McGill University. The monkey was provided by the kindness of Dr. J. Craigie, of the School of Hygiene of the University of Toronto.

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VISCERAL LESIONS IN POLIOMYELITIS *

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While poliomyelitis is the subject of much research, and many studies on its etiology, its epidemiology, and its immunologic and serologic aspects are on record, recent gross and histologic studies of organs other than the brain and spinal cord are singularly lacking. An exception is the recent report of Dublin and Larson [1] upon the results of 12 post-mortem examinations made during the epidemic of 1940 in Pierce County, Washington. They found, in addition to the lesions in the brain and spinal cord, lymphoid hyperplasia of the intestinal mucosa and mesenteric nodes, acute myocarditis twice, and bronchopneumonia in three instances, with abscess formation in one. Apparently one of these instances of myocarditis had been previously reported by Larson [2] (1941). Hemorrhagic pulmonary edema was outstanding in three cases. The liver regularly disclosed some degree of hydropic change and, in some instances, fatty degeneration.

This study is based on post-mortem examinations of 17 patients who died of clinically diagnosed poliomyelitis during the recent Chicago epidemic. The duration of the disease varied from 3 to 8 days. The diagnosis of bulbar and spinal poliomyelitis was made in 13 cases. Bulbar poliomyelitis was diagnosed four times, and in one of these encephalitis was also recognized clinically. There were 12 children under 14 years of age, 2 adolescents, 16 and 17 years old, and 3 adults, 20, 23 and 28 years old. All patients had received various amounts of either convalescent human serum or pooled normal human serum. Six patients received sulfadiazine. A respirator was used for 4 patients.

The outstanding changes encountered at autopsy were as follows: The hearts were dilated and often soft and flabby. The mural and valvular endocardium was smooth. The papillary muscles and chordae tendineae were flattened. The myocardium invariably was pinkish gray or brownish gray and of boiled appearance with loss of its normal architecture. Petechial hemorrhages were often encountered in the endocardium and epicardium. In one instance they were abundant and covered practically the entire visceral pericardium.

The lungs grossly disclosed several findings. In ten instances marked edema and acute hyperemia were encountered. Slight bronchopneumonia was detected three times and severe bronchopneumonia twice. In

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six lungs in which either edema and hyperemia or no gross change was encountered, significant lesions were discovered on microscopic examination. The bronchi and trachea showed no lesion in these instances. In some of these latter patients, attending physicians had diagnosed a mucous plug in the bronchus. It was surprising that such a mucous plug was actually encountered at necropsy only once. Moderate emphysema was found twice in children who, because of difficulty in breathing, had been placed in a Drinker respirator. Edema and hyperemia also were found in these lungs. Only the lungs of 1 patient were free from pathologic change.

Cloudy swelling of varying degree was invariably present in the liver and in the kidneys. In the latter, however, passive hyperemia was the outstanding finding.

The intestinal tract disclosed most commonly a marked hyperplasia of the lymph follicles and of Peyer's patches. There was also, in these instances, a hyperplasia of the peritoneal lymph nodes. Occasionally, hyperplasia of the Peyer's patches was so severe as to resemble that seen in the stage of medullary swelling of typhoid fever. It may be noteworthy, however, that the hyperplasia was not observed in 3 patients, the oldest in this series, who were 20, 23 and 28 years old, respectively.

Cerebrospinal lesions may be summarized only. Twelve patients disclosed inflammatory lesions in the brain itself in addition to the lesions in the medulla oblongata and spinal cord. The regions surrounding the ventricles were most commonly involved. These changes were particularly common in the vicinity of the fourth ventricle. The cerebellum was also frequently involved. The most outstanding site was the dentate nucleus. The spinal cord disclosed grossly recognizable, minute and larger foci of hemorrhage within the gray matter. These lesions were encountered always, but not exclusively, in its anterior horn. Often the posterior portions were also involved but usually to a lesser degree. The changes were marked in the cervical and often in the upper thoracic cord, and became less pronounced or were absent in the lower thoracic segment, but again were more prominent in the lumbar region.

Microscopic examination of the myocardium disclosed myocarditis in ten instances. In two the inflammatory changes were very marked and diffuse, in six they were more or less localized, and in two hearts the changes were only slight. In the first two hearts the changes were so severe that there seemed to be a massive extravasation of leukocytes. Many polymorphonuclear leukocytes were encountered and also lymphocytes. The proportions of these two cell types varied markedly

in the various sections. Red blood corpuscles also were present in the inflammatory exudate. Many heart muscle fibers were compressed by the exudate and appeared thinned out, while others were the seat of cloudy swelling. There was no evidence of necrosis. In the six instances in which the inflammatory exudate was localized, it was seemingly confined to the interstitial tissue. This exudate consisted of lymphocytes and only a few polymorphonuclear leukocytes. Endothelial leukocytes were rarely encountered. Both the right and left ventricles were involved. Often the inflammatory changes were most marked in the vicinity of epicardial hemorrhages.

The aortas, which grossly had disclosed no noteworthy changes, were the seat of microscopic alterations in three instances. There was an edema-like material seen in the media separating the elastic lamellae and sometimes interrupting their course. Often this material had a pinkish, smudgy, fibrinoid appearance. Here and there an occasional lymphocyte could be made out. Neither the intima nor adventitia showed any change.

In the lungs, the lesions encountered grossly could easily be verified microscopically. Thus, edema and hyperemia were very often seen, and bronchopneumonia in varying degrees was observed five times; in three it was very slight. However, in six additional instances definite interstitial pneumonia could be demonstrated. It was noted that the smaller bronchi in these instances disclosed the presence of lymphocytes and of polymorphonuclear leukocytes within the submucosa, whereas the lining cells of the mucosa were intact. The lumina of these bronchi did not contain an appreciable number of inflammatory cells. The only noteworthy change in the mucosa was a thickening of the basal membrane. It could be demonstrated that the inflammatory cells from the submucosa of the smaller bronchi extended either within or along minute vessels into the alveolar septa, which often were crowded with lymphocytes, polymorphonuclear leukocytes and red blood corpuscles. Here and there some of these inflammatory cells extended into the adjacent alveoli. Occasionally a thin eosinophilic, homogeneous material could be made out lining the inner wall of the neighboring alveoli. The larger bronchi and the trachea also had a thickened basal membrane of the mucosa and, sometimes, varying numbers of inflammatory cells throughout their walls. The lumina rarely contained an accumulation of mucus. As stated before, a mucous plug was encountered grossly only once. The lining epithelial cells of the trachea and larger and smaller bronchi were carefully examined for inclusion bodies. In not a single instance, however, were inclusion bodies encountered.

The solitary follicles and Peyer's patches in the lower ileum and cecum were sometimes enormously hyperplastic. The hyperplasia involved the germinal centers. There was, however, no evidence of necrosis in these areas. The adjacent regions of the intestinal tract showed no inflammatory changes whatsoever. The spleen and mesenteric lymph nodes also disclosed follicular hyperplasia with conspicuous germinal centers. In the midst of some of the latter, foci of necrosis were discernible. No other change could be detected. In the remaining viscera there were no noteworthy histologic lesions.

The microscopic changes in the brain and spinal cord will be enumerated only, since there are many reports on record describing these changes and this study contributes no new details. As noted grossly, the more severe lesions in the cerebrum and cerebellum were found in the vicinity of the ventricles, particularly about the fourth ventricle. The dentate nucleus was frequently involved. Many ganglion cells disclosed degenerative changes with absent nuclei and with Nissl's granules obscured. Lymphocytes and occasionally—in some instances, however, more pronounced—polymorphonuclear leukocytes were encountered perivascularly. Here and there small accumulations of proliferated microglial nuclei could be seen. In the spinal cord the changes in general were not confined to the anterior portions of the gray matter, though they were most conspicuous in these areas. The changes consisted of marked hyperemia, minute foci of extravasation of red blood corpuscles, and perivascular infiltrations of lymphocytes, mainly, and a few polymorphonuclear leukocytes. Hemorrhage never constituted a predominating feature. In the more severe examples the inflammatory cells were found diffusely throughout the gray matter. Here and there ganglion cells, evidently necrotic, were found in the midst of the inflammatory exudate. Endothelial leukocytes, though in general not numerous, seemed to be relatively more frequent in these areas. Often the regions surrounding the central canal were the seat of an inflammatory reaction. Although the white matter of the cord was rarely involved, small infiltrations of lymphocytes occasionally were observed. The meninges also contained inflammatory cells more or less confined to the perivascular areas, particularly in the more severe instances.

Comment

Aside from the known lesions in the brain and spinal cord, the more outstanding changes encountered in these 17 cases of poliomyelitis were myocarditis and interstitial pneumonia.

Myocarditis was found ten times. It must be stressed that in some instances myocarditis was recognized in the first few sections exam-

ined, but in the majority a number of blocks had to be examined before evidence of myocarditis was found. From this study it seems evident, and it apparently also holds true for many other infectious diseases, that myocarditis will be encountered frequently if the myocardium is examined histologically with the specific purpose of either ruling out or establishing the presence of inflammatory alterations.

Since myocarditis is known to occur in instances of pneumonia [3] or as a result of sulfa drug medication,[4] the question arises as to whether or not the myocarditis in poliomyelitis may be related to these two factors. However, the clinical records disclosed that 4 of the 10 patients did not receive sulfa drugs, though the remaining 6 did receive sulfadiazine. Only 4 of the patients showed at necropsy evidence of pneumonia. All patients were treated clinically with either pooled serum or serum taken from patients who had recovered from poliomyelitis. It does not seem likely that the myocarditis signifies a so-called serum reaction, since an exudative reaction following administration of homologous human serum is allegedly very rare.[5] None of the patients showed any clinical signs or symptoms which possibly could be the result of an abnormal serum reaction. It should be pointed out that the patient who had the most severe myocarditis had received pooled normal serum and not convalescent serum. In this series there was no relationship between the presence or absence of myocarditis and the type of poliomyelitis, whether principally bulbar or spinal.

An instance of polioencephalomyelitis associated with optic neuritis and myocarditis was reported as early as 1913 by Hertz, Johnson and Depree.[6] However, this was a clinical observation, and no autopsy was performed to verify the clinical diagnosis of myocarditis. Wile and I [7] recorded myocarditis in poliomyelitis in six of seven hearts which were specifically examined histologically for myocarditis. Peale and Lucchesi [8] found myocarditis of some degree on histologic examination in seven of nine hearts of patients with poliomyelitis. Larson [2] stated that since neither bronchopneumonia nor any other source of infection was found in his patient as the cause of the myocarditis, it may be theorized that the myocardial involvement was produced by the virus of poliomyelitis.

The sudden death of some of these children can easily be explained by the myocarditis. Hassin [9] described a child with poliomyelitis, who presented a clinical picture of Landry's paralysis with microscopic inflammatory changes within the muscles. The sudden death of this child was attributed to involvement of the myocardium.

Another interesting finding in this series was the presence of an interstitial pneumonia. It was observed that the inflammatory exudate

spread from the submucosa of the smaller bronchi into the peribronchial tissues and thence into the alveolar septa. The inflammatory cells were lymphocytes and polymorphonuclear leukocytes, sometimes the former being more numerous. This interstitial involvement of the lung was often found in conjunction with a severe hyperemia. It is noteworthy that this type of pneumonia was encountered in those instances in which there was much cyanosis clinically, and when the attending physician had expected a mucous plug within the trachea or bronchi. This pneumonia resembled so-called atypical pneumonia. However, the inflammatory cells were not principally monocytes as in atypical pneumonia. Though no inclusion bodies were found anywhere in the lungs, it is possible that a virus may have caused this pneumonia. It might be of interest in future cases to conduct virus studies with material taken from the lungs of patients who die from poliomyelitis.

Hyperplasia of the intestinal lymph follicles and Peyer's patches has often been observed and originally suggested the gastrointestinal tract as the portal of entry.[10] However, the hyperplasia of the solitary lymph follicles and of the abdominal lymph nodes differed in no way from that seen in many acute infectious diseases in childhood. Microscopically there was no evidence of acute inflammatory change in the vicinity of the lymph follicles. There were, however, occasional small foci of necrosis of germinal centers. It is also noteworthy that the older patients in this series did not have this lymphoid hyperplasia.

Summary

The findings at necropsy of 17 patients dying from poliomyelitis are recorded. The changes in brain and spinal cord were not unusual and are given only cursory mention. In ten instances myocarditis was found. It was detected only on microscopic examination and varied greatly in extent and severity. Often it was noted in the vicinity of minute or larger epicardial or endocardial petechial hemorrhages. Myocarditis was apparently in no way related to pneumonia or to therapeutic measures. The sudden death of some of these patients may be attributed to the myocarditis. Interstitial pneumonia was encountered six times. It is possible that the pulmonary lesions were caused by a virus. Bronchopneumonia was present five times. Hyperplasia of the lymph follicles and Peyer's patches, although almost constant in children, is not an important feature and is not characteristic of poliomyelitis.

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For additional references that may be consulted, see also Infantile Paralysis: A Symposium Delivered at Vanderbilt University, April, 1941. Published by the National Foundation for Infantile Paralysis, Inc., 120 Broadway, New York City.

[*Illustrations follow*]

DESCRIPTION OF PLATES

Plate 16

Fig. 1. Heart with inflammatory cells principally in the interstitial tissue. Most of the cells are lymphocytes but a few polymorphonuclear leukocytes are present also. Iron-hematoxylin and eosin preparation. × 150.

Fig. 2. Severe degeneration of myocardial fibers with diffuse infiltration of inflammatory cells, mainly polymorphonuclear leukocytes. Iron-hematoxylin and eosin preparation. × 130.

Fig. 3. Myocardium. Inflammatory cells are located principally in the interstitial tissue. The inflammatory cells are both lymphocytes and polymorphonuclear leukocytes. Iron-hematoxylin and eosin preparation. × 80.

Fig. 4. Heart with heavy infiltration of polymorphonuclear leukocytes and lymphocytes. Iron-hematoxylin and eosin preparation. × 130.

Fig. 5. A field similar to that shown in Figure 4. Iron-hematoxylin and eosin preparation. × 200.

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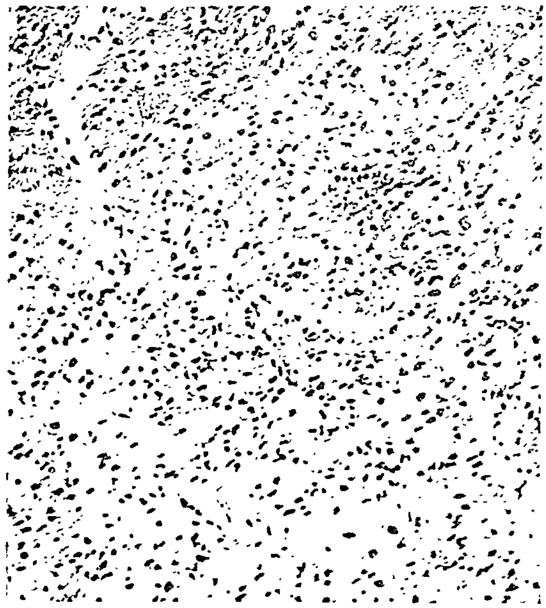
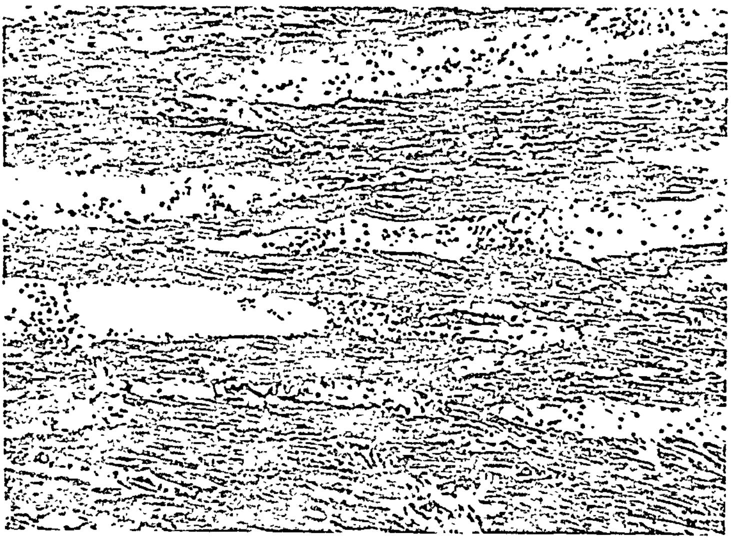
 2

3 

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Visceral Lesions in Poliomyelitis

Plate 17

Fig. 6. Aorta, showing edema-like material separating the elastic lamellae and the presence of few inflammatory cells. Iron-hematoxylin and eosin preparation. $\times$ 120.

Fig. 7. A field similar to that shown in Figure 6. Iron-hematoxylin and eosin preparation. $\times$ 180.

Fig. 8. Trachea, showing hyperemia and the presence of lymphocytes and a few polymorphonuclear leukocytes within the submucosa. Iron-hematoxylin and eosin preparation. $\times$ 140.

Fig. 9. Small bronchus, showing infiltration of the submucosa principally with polymorphonuclear leukocytes, and extension of the inflammatory cells between the muscle fibers. Iron-hematoxylin and eosin preparation. $\times$ 150.

Fig. 10. Interstitial pneumonia. Lymphocytes and a few polymorphonuclear leukocytes are present within the septa, while the alveoli are empty. Giemsa preparation. $\times$ 110.

Fig. 11. A field similar to that shown in Figure 10. Giemsa preparation. $\times$ 180.

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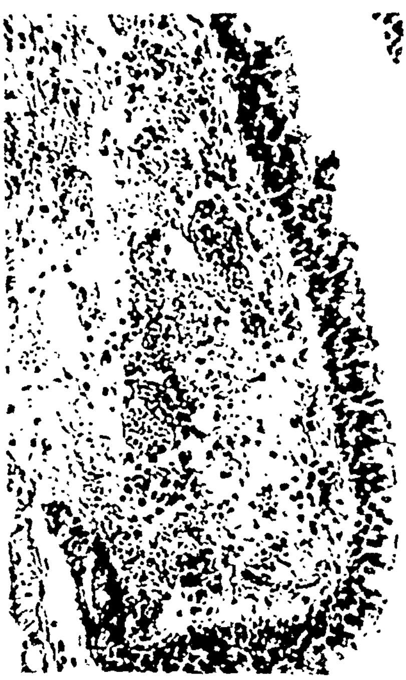

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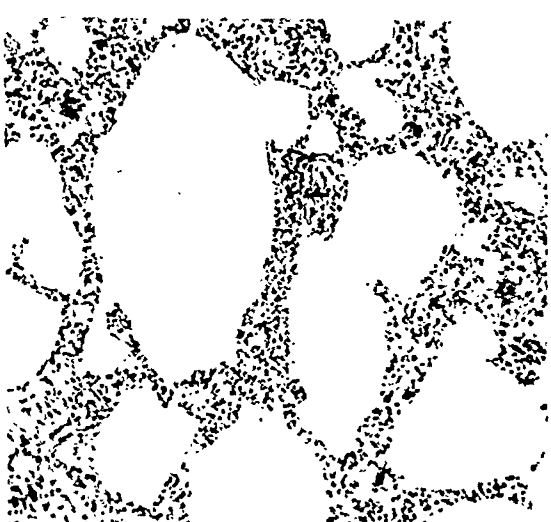

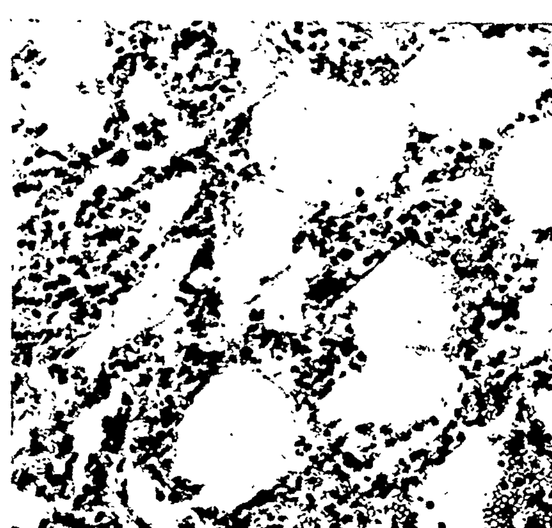

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Visceral Lesions in Poliomyelitis

GASTRIC ULCER IN SWINE*

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Ulcers, that from their gross and microscopic appearance are strikingly similar to the ulcers of the stomach in man, have been observed in the stomachs of swine. A report of our findings on gastric ulcer in swine is submitted.

The cases of stomach ulcer which I have studied were all found in connection with a necropsy service on animals. The series from which they were collected includes a total of 754 swine. Eighteen (2.38 per cent) of these pigs had one or more typical ulcers in the stomach. In general, the gross morphology of the ulcer was that of a rounded, circumscribed excavation in the mucous membrane. The floor of the ulcer was flattened and below the surface of the surrounding mucosa. In 2 cases the floor extended into the muscle coats and the serosa over this area was thickened. The walls were usually steep. The rim or border overhung the wall in 1 case. A zone of inflammation surrounded the lesion in most cases, and, in 2, bleeding from the lesion was very evident. Microscopic examination shows that the mucous membrane stops at the ulcer crater in most of the cases. Where the muscle coat was involved, the muscle seemed to be retracted and dense fibrous tissue invaded the area. The floor was covered with granulation tissue.

A single or solitary ulcer occurred in 10 cases and 2 or more (2 to 15) ulcers were present in the other 8 cases. In 5 of the cases in which a solitary ulcer was present, the lesion was situated in the pyloric gland region. The ulcer was situated in the fundic gland region in 2 cases and in the cardiac gland region in 2. No record is available for 1 case. For the most part, the ulcers were on the greater curvature of the stomach. The largest ulcer measured 6 cm. in diameter. When more than a single ulcer was found, their particular location was usually not recorded. However, in one instance, 4 ulcers were present in the fundus, and in another, 6 were in the pyloric region.

Unfortunately, the exact age of our animals is not known, but a reasonably accurate approximation of the age can be made in most instances. The youngest pig with gastric ulcer was a Duroc-Jersey male between 6 and 7 weeks old. In this pig, there were 4 ulcers in the fundic gland region and each measured about 1.5 cm. in diameter. The state of nutrition in this animal was very poor. It was thin, dehydrated and weak. The history on the group from which this pig

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was chosen as representative revealed that a nutritional or iron-deficiency anemia had been prevalent about 2 or 3 weeks previously. A diagnosis of post-anemic cachexia with secondary gastric ulcers was made. A history of what probably was nutritional anemia was obtained on 4 other cases with gastric ulcer. These pigs, however, were between 10 and 14 weeks of age. Bronchopneumonia was considered to be the principal cause of the illness or death in 5 pigs that showed stomach ulcers at necropsy. In none of these cases was the illness ascribed to the gastric lesion. In 2 animals, the ulcers appeared to be healed or healing. The age of these pigs was estimated to be from 5 to 8 months. Hog cholera was the cause of the illness or death in 6 cases. In addition to the excavating ulcer in 1 of the latter, there were present a few ulcers of the "button" variety more commonly seen in this disease when ulceration occurs. These pigs were all more or less acutely sick and came from herds in which others were sick or dead. The youngest pig in this group was about 60 days of age and the oldest about 7 months. In 2, the chief gross pathologic process accountable for their clinical condition was an extensive diphtheritic and necrotic colitis (generally described as infectious enteritis in veterinary medical literature). One was a female between 4 and 5 months of age which weighed only 30 lbs.; the other was a female of 11 months that weighed 180 lbs. The gastric ulcers in these 2 cases presented evidence of advanced healing, the mucosa dipping down into the crater and covering part of the floor whereas the inflammation in the lower bowel was more acute.

Comment

The occurrence of ulcers in the stomachs of swine, which from their morphologic characteristics are not unlike the gastric or "peptic" ulcers in man, has been observed on 18 occasions. In not a single instance was their presence suspected from any signs or symptoms displayed by these animals. If a free incision of the stomach had not been made and the mucosal surface everted and freed of ingesta, several of these ulcers would have been missed. This disease in swine, based upon the series studied, shows an incidence of 2.38 per cent. It is to be noted that at least 98 per cent of the animals comprising the series were pathologic cases submitted to our necropsy service for the express purpose of ascertaining the cause or nature of the disease responsible for their condition. Even though the viscera of millions of swine are handled by veterinarians in the meat inspection service of the Federal Government each year (46,520,000 hogs slaughtered under Federal inspection in 1941 [1]), no information on the incidence of gastric ulcer in the general swine population is available. The reason

for this is that the stomachs are not incised and the mucosal surface not examined at the "bench" unless some obvious lesion on the serosa warrants the investigation.

The group of pigs with gastric ulcers is much too small to establish the incidence as to sex. Eight were males. That all were pigs less than 1 year of age may be of some significance in respect to the cause. In this connection, I wish to point out that in age the pigs in the entire series (754 swine) ranged between stillborn and nearly 5 years old. The greatest number, however, were between 5 and 11 months of age. Typical excavated ulcers of the gastric mucosa were found in a pig scarcely more than 1 month of age, and also in a few others that were less than 4 months of age. The presence of an ulcer in such young animals suggests that there may be a causal relationship to the occurrence of nutritional anemia. Nutritional anemia is not an uncommon disturbance of suckling pigs that are born in the late winter and early spring in this part of the country. No satisfactory explanation for the specific and definite cause of these ulcers in swine is forthcoming at this time.

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1. Agricultural Statistics, United States Department of Agriculture, 1942, Table 520, p. 403.

AN OVINE MONSTROSITY

(Cormo-melodidymi Dipygus Bidorsualis) *

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Since teratology has been placed on an embryological basis, much of the mystery and superstition concerning the development of monsters has been eliminated. By scientific study of terata, it is shown that anomalies are the result of irregularities arising at any stage of the developmental period.

According to Arey: [1]

> "The incidence of major malformations in the newborn [human] is about 1:165, the ratio is higher in aborted fetuses, while the inclusion of minor anomalies would raise the frequency for both groups.
>
> "Through the perversion of developmental processes, embryology and pathology find a common meeting ground; indeed, teratogenesis may be characterized as pathological embryology."

Stockard [2] believes that developmental inhibition or arrest is the single causal factor in most abnormal development, including twinning. He inhibited the developmental process by lowering the temperature or reducing the oxygen supply, the resulting type of deformity being dependent solely on the precise moment when the interruption occurred.

Double monsters, according to present theories, may originate from two ova which are united, or may be produced by division of a single ovum.

Description of the Specimen

The monstrosity was sired by a Shropshire ram, and its dam was a grade Shropshire ewe, 3 years old. Parturition was of long duration but accomplished without assistance. The gestation period was approximately 150 days, and all organs were apparently fully developed. Death occurred near or at the time of birth.

According to Gurlt's classification of monstrosities as presented by Fleming,[3] the specimen is designated as "Cormo-melodidymi dipygus bidorsualis." It consisted of two fetuses of approximately equal size forming a symmetrical monster having one head, and four thoracic and four pelvic limbs. This monocephalian monstrosity was joined at the ventral surface from the umbilicus cephalad, and had the liver, spleen, pancreas, stomach, part of the intestine, lungs and heart in common. Complete separation of the equal conjoined twins occurred posterior to

* Received for publication, April 3, 1944.

the umbilicus, while the union of the vertebrae became complete at the axis to form one atlas which articulated with the single head.

Anatomical Detail

The Skeleton. Deep dissection and, finally, maceration were necessary to demonstrate the one complete skull which articulated with the single atlas. The axes and the third cervical vertebrae were paired, but united to their fellows of the opposite side by their transverse processes. The vertebral formula of each lamb, counting the single atlas with both, was C. 7, T. 13, L. 6, S. 4, Cy. 15.

Two sterna were present, actually assuming a right and left position to form a portion of the lateral walls of the common thoracic cavity. Thirteen pairs of ribs were present, with seven sternal and six asternal.

Complete skeletal development was shown by the four thoracic and four pelvic limbs.

Digestive and Respiratory Systems. Both the hard palate and the soft palate were cleft to a severe degree. Figure 3 shows them to be only slightly developed. Often cleft palate is associated with hare lip, but there was no evidence of this condition. The tongue was thickened dorsoventrally so that its dorsal surface fitted into the cleft of the palate.

Not only did the oral and nasal cavities communicate, but also it was found that the respiratory and digestive tracts were developed as a common tube extending from the oral and nasal cavities to a point midway between the base of the heart and the diaphragm. This esophago-tracheal tube with a single lumen contained plates of cartilage in its dorsal and ventral walls; no complete rings were developed. A horizontal membrane divided the lumen of the incompletely developed larynx into dorsal and ventral parts. The posterior displacement of the larynx to a point ventral to the third and fourth cervical vertebrae was unusual. The esophago-tracheal tube terminated near the center of the diaphragm in four bronchial apertures and three esophageal openings. The right bronchi supplied the two lungs of the left lamb similarly. A central digestive aperture entered the centrally located omasum, while an aperture on each side of this central opening entered bilateral abomasa.

From the left abomasum, the intestine coiled to the left, occupying mainly the abdominal cavity of the left twin until it terminated posterior to the cecum in two branches. Each branch of the intestine continued as the normally arranged colon to the rectum of its respective lamb. Each anus was perforate.

A single large liver extended across the abdominal cavity in relation to the single complete diaphragm. The liver received a large portal vein and two umbilical veins. Inside the liver, the portal and umbilical veins united to form a venous sinus, from which the blood entered the ductus venosus.

A single spleen was observed in the left lamb and one pancreas was found in the right lamb.

Urogenital System. The urinary system was normal and complete for each individual. Both lambs were females, normal genitalia being present in the left lamb, but incomplete development only in the right. The right ovary, uterine tube and the uterus of this lamb presented normal development, but the left uterine tube and horn were absent. The left ovary was found in the broad ligament, having no tubal connection with the uterus.

Circulatory System. Two umbilical veins passed from the common umbilicus to the liver where they anastomosed with each other and formed a venous sinus by confluence with the single portal vein. From the venous sinus, the blood passed through the ductus venosus to the very large posterior vena cava. The vena cava deviated to the side of the left lamb but entered the right atrium. Further dissection revealed a small vein on the right side extending from the iliac vein (and receiving the renal, uterine and lumbar tributaries) to the junction of the right jugular vein and the anterior vena cava.

A single heart supplied both individuals, but the pulmonary artery and aorta were so developed that contraction of either ventricle would force blood to both lambs. The right and left ventricles were of almost equal size. Persistence of the foramen ovale was noted.

The accompanying diagram (Text-Fig. 1) illustrates the peculiar arrangement of the arterial system in the cardiac region. The pulmonary artery turns around the aorta, gives off branches to supply the lungs of both lambs, and continues posteriorly as an aorta for the right lamb. It did not give off an anterior mesenteric artery. The right brachial artery branches from the pulmonary artery. An anastomosing vessel connects the arch of the aorta with the arch of the pulmonary artery.

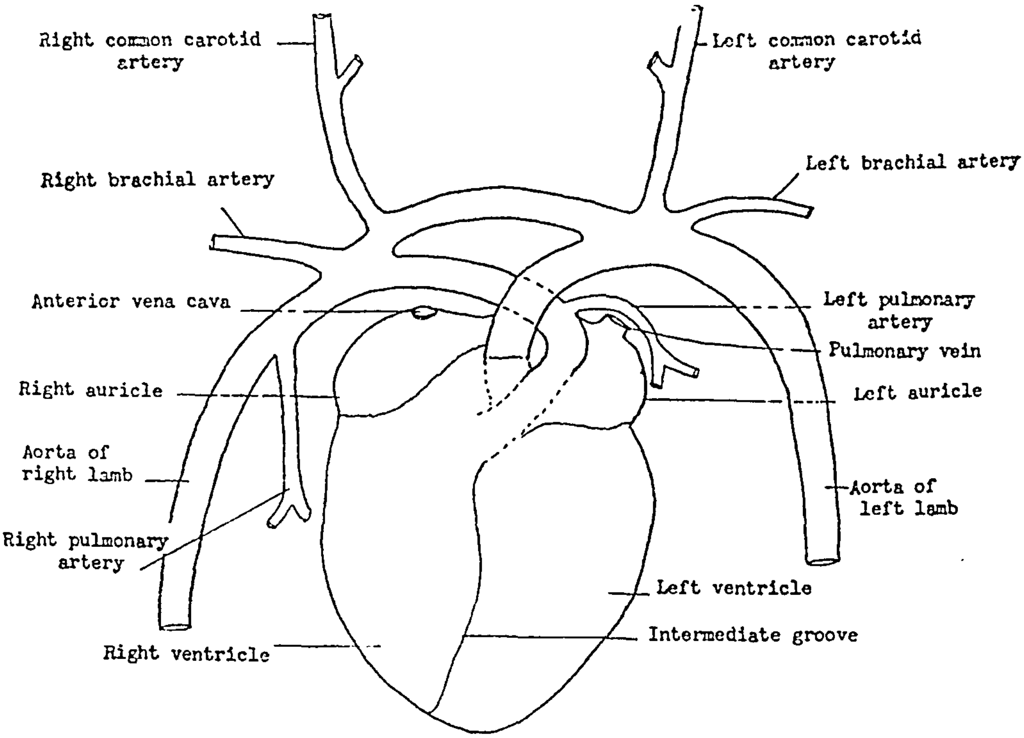
The true aorta arising from the left ventricle gave off coronary arteries and a brachiocephalic trunk, and continued posteriorly in the left lamb in the usual manner.

Four umbilical arteries were noted; two from the external iliac arteries of each twin.

Nervous System. A dual nervous system existed posterior to the

bifurcation of the spinal cord at the second cervical vertebra. A single spinal cord entered the atlas and continued anteriorly to the single brain.

Monsters commonly die at birth due to anomalies which interfere with normal postpartum body functions. In this instance, death may



Text-Fig. 1. Diagram of heart and arterial branches.

have been due to collapse of the esophago-tracheal tube since the tracheal rings were incompletely developed, or to the malformed blood-vascular system which was so arranged as to supply a prenatal circulation but was inadequate for postnatal circulation of blood through the lungs.

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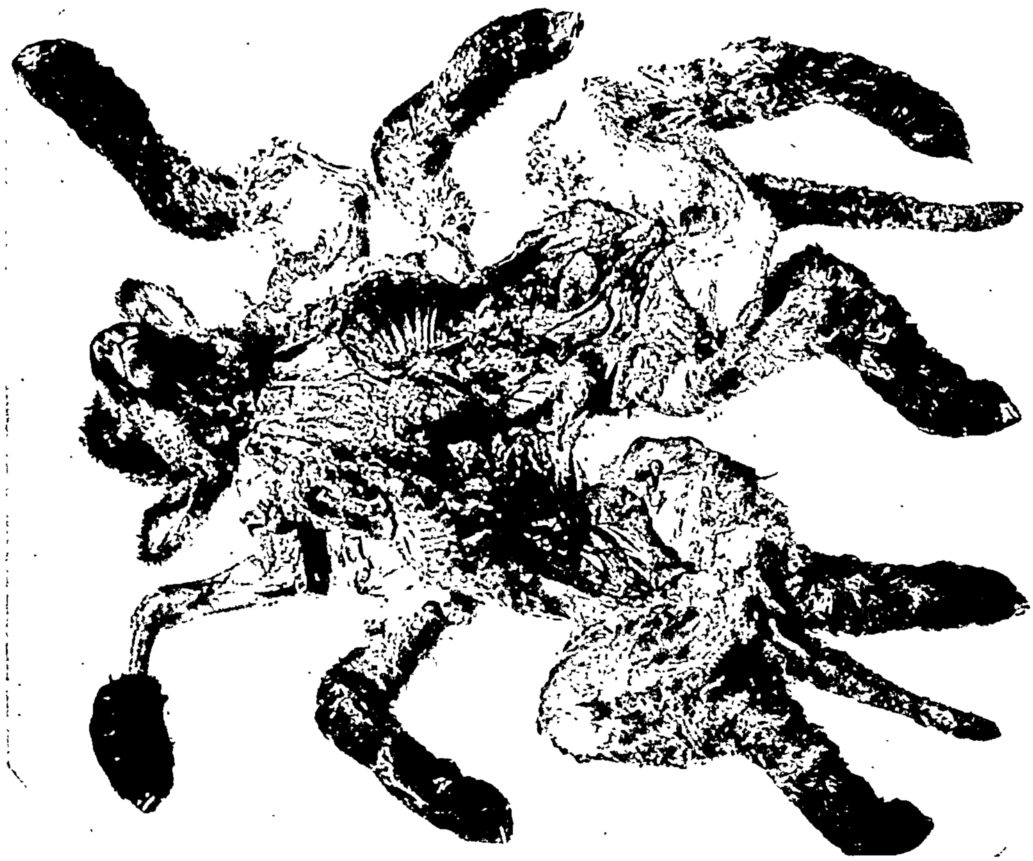
DESCRIPTION OF PLATES

PLATE 18

FIG. 1. Ovine cormo-melodidymi dipygus bidorsualis. Dorsal view.

FIG. 2. Ovine cormo-melodidymi dipygus bidorsualis. Ventral view.



Goss and Cole

Ovine Double Monster

PLATE 19

FIG. 3. Cleft palate and dissected cardiac region.

3

Goss and Cole Ovine Double Monster

EXPERIMENTAL SILICOSIS PRODUCED WITH THE ASH FROM HUMAN SILICOTIC LUNGS *

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The pathologic effects on human lungs of fine silica particles, inhaled in industries where silica hazards exist, are well known. What happens to the silica particles in the body and whether or not they are inactivated for further silicotic reactions apparently has not been determined.

It is now generally accepted that the fine crystalline silica (SiO_2) particle is the toxic agent and that the rate of fibrous reaction which it stimulates depends primarily on its solution in the form of colloidal silicic acid. The freer it is from contamination and the smaller the size of the particles, the more promptly the tissues react to it, and the more rapid and extensive is the silicosis which results. It is known further that the fibrosis of silicosis does not cease when the dust inhalations are stopped but that the process may continue for many years without the stimulation of fresh particles. Mixtures of silica with other dusts such as carbon, iron and aluminum affect the toxicity of silica and modify the severity of, or prevent, tissue responses. When silicotic lungs are analyzed chemically a large portion of the residue is made up of silicates, but so far it is not possible to tell whether the silica was changed in the tissues or whether it was originally inhaled as a mixed dust in approximately the same proportions as those recovered in the chemical extracts. This report deals with the results of injecting the chemically recovered ash of silicotic lungs into experimental animals as second hosts.

For some years, one of us (Taylor) has been making chemical analyses of lungs sent to the laboratory for the diagnosis or exclusion of silicosis. We have not depended upon chemical data alone. We have made gross and microscopic examinations, supplemented by incinerated sections, and have supported our conclusions with chemical data whenever possible.

There exists a considerable divergence of opinion concerning the value of chemical analyses in the diagnosis of silicosis. King and Belt [1] reviewed the subject thoroughly and Belt, Irwin and King [2] reported a series of analyses in which they found as much SiO_2 in lungs of nonsilicotic miners as they found in some of those with massive silicofibrosis. McNally [3] considered that anything over 0.2 per cent indi-

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cated excessive exposure to silica although he cited exceptions to this rule in several of his cases. Sweany, Porsche and Douglass [4] considered anything over 0.2 per cent to be abnormal in the lungs. They found that lymph nodes sometimes contained 5 per cent without a record of abnormal exposure. Gardner and Redlin [5] made analyses of 150 lungs for silica content: 58 were from persons with no known exposures to silica, 20 were from subjects with histories of exposure but with no evidence of silicosis, and 72 were from persons who had both a history of exposure and classical lesions. In 32 instances there was no correlation between the content of silica and the lesions present. They concluded that there could be no exact correlation between free silica and pathologic reactions; that free silica may be high and its irritating influence counteracted by inhibiting minerals; and that the true relative values may be lowered by an excess of newly formed tissues in the silicotic lung. We would agree, in general, with this statement although our results so far have coincided with the findings of Sladden [6] in that lungs which have advanced silicofibrosis yield constantly more than 1 per cent of silica in dried lung.

There are three precautions that must be observed in the extraction of the ash of silicotic lungs if the results are to be consistent with the degree of dusting that has taken place. Since the silica particles are partly concentrated in the lymph nodes (Belt, Irwin and King [2]) and since the tissues may be unevenly diluted by exudative processes, it is important that whole lungs, as nearly as possible, be used for the recovery of the dust. Only the lowest temperature consistent with reasonable speed of destruction of the organic matter may be employed. Otherwise the physical state of the particles may be altered and with it the physiologic activity (Nagelschmidt and King [7]). Finally, the ash may be extracted only with dilute acid (1.0 N HCl) and for the minimum time necessary for removal of iron and phosphate deposits that tend to cement the particles together as well as to interfere in the determination of the free silica (Taylor [8]). It is also helpful, when the two lungs differ in the pathologic processes present, to determine the silica present in each lung so that they may be compared on the basis of the 47/53 ratio of normal left and right lung weights.

Since the residues of our chemical extracts were small in amount we decided to test them by the injection methods along lines used by Gye and Purdy, Gardner and Cummings, and Miller and Sayers. In 1922, Gye and Purdy [9] demonstrated the toxicity of colloidal silica when injected subcutaneously, intraperitoneally and intravenously into guinea-pigs and rabbits. They found that large daily doses of from

30 to 32 mg. caused death in rabbits in from 2 to 4 days, and that smaller doses injected into rabbits at weekly intervals produced cirrhosis of the liver. Gardner and Cummings,[10] in 1933, developed an injection test for the study of the effects of dust particles, based upon repeated intravenous injections of small doses suspended in normal salt solution. Miller and Sayers,[11] in 1934, used an intraperitoneal technic for testing tissue reactions to dusts of unknown toxicity. They classified the reactions as inert, when the particles provoked a transitory reaction which later disappeared; absorptive, when insoluble dusts produced a minimal amount of inflammation in which the particles were phagocytized and remained in the tissues without an increase in the reaction as time went on; and proliferative, when the dusts contained enough silica, either alone or in association with other dusts, to cause progressive proliferation resulting in the production of fibrous nodules.

Experiments

The general plan of the experiments provided for tests by animal injection of four samples of ash recovered chemically, and a control series in which pure crystalline silica was injected. The cases were selected to include one in which the insoluble dust was low in total silicates and without free silica, two cases in which mixed dusts containing free silica were recovered, and one case from which silicates containing excessive amounts of free silica had been obtained.

In each group three rabbits were given intravenous injections on alternate days of a 1 per cent suspension of the respective ash and three guinea-pigs received injections of 2.5 cc. of a 1 per cent suspension intraperitoneally. As the experiments proceeded, some slight variations in the amounts used became necessary. The silica control group was arbitrarily called group III and will be discussed first.

Control Group III

Rabbits. One rabbit received 6 intravenous injections of crystalline silica on alternate days and died on the 13th day. Another rabbit received 8 injections and died on the 17th day immediately after the last injection. The findings in these two rabbits were limited to nodular thickenings at the sites of injection along the ear veins, to subintimal infiltration in the veins and to accumulations of phagocytes in the liver, sinuses and spleen.

The third rabbit survived 8 injections in the ear veins and lived for 23 months. Grossly, the ear veins presented bead-like nodules, the pleura showed minute black spots, the spleen and kidneys were prac-

tically negative, and the liver showed subcapsular depressed scars and presented the typical picture of cirrhosis. The deep wrinkling of the capsule was present uniformly over the liver save for the spigelian lobe which was replaced by yellow fibrous tissue.

Microscopically, the pulmonary alveoli contained many black-dust phagocytes which tended to collect in the perivascular and peribronchial lymph nodes. Some of the pulmonary veins were thickened and their walls infiltrated with hyaline connective tissue, the meshes of which contained silica phagocytes. Occasional vessels had nodular thickenings in their perivascular zones made up of agglomerations of phagocytes filled with minute silica crystals. In several instances the lymphoid tissue was displaced by central nests of phagocytes. The liver was cirrhotic. The periportal zones were increased in size, thickened, devoid of liver cells and showed hyperplasia of the bile ducts. There were nodular zones made up of fibrous tissue which contained collections of phagocytes. The spleen contained nodules of phagocytes with giant cells and in some instances the centers were necrotic. The kidneys showed a moderate degree of diffuse cortical fibrosis. There were nodules in the ear veins at the sites of injection, composed of collections of subintimal phagocytes filled with particles. The nodules were large and almost obliterated the vascular lumina.

Incinerated sections of the liver showed diffusely distributed agglomerations of silica particles in the fibrous areas. In the ear veins and spleen the agglomerations were much more abundant and more closely arranged.

Guinea-Pigs. Of the three guinea-pigs that received the intraperitoneal injection of 1 per cent silica suspension, one died after the third dose. Post-mortem examination showed small nodules in the muscle at the sites of injection, in the mesentery, omentum, between the intestinal loops and under the liver. The second and third guinea-pigs died at 6 and 18 months after the intraperitoneal injections from obstruction of the bowels due to peritoneal adhesions. They presented similar gross and microscopic changes. The peritoneal lesions were large, necrotic, partially calcified and contained great numbers of multinucleated foreign body giant cells. The spleens showed hyperplasia of the germ centers and collections of silica phagocytes surrounding the germ centers. In one of the animals the splenic veins were filled with crystalline particles as well as some fine, brown, particulate material. Throughout the liver there were nodular collections of phagocytes but no cirrhosis. Special stains for fibrous connective tissue showed that the nodules had formed their own stroma. The lungs of

both animals contained alveolar phagocytes filled with black pigment, probably due to inhaled pigment from the air. The perivascular lymphoid tissue contained some collections of phagocytes filled with black pigment, and several small perivascular lymph nodes contained typical silica tubercles with collagenous nodules suggesting the yarn-ball-like arrangement seen in human cases of silicosis.

Summary of Control Groups. All of the rabbits in the control series showed perivascular phagocytes and the progressive type of silica nodules along the ear veins and in the liver and spleen. The rabbit that lived 23 months showed advanced cirrhosis of the liver. The guinea-pigs that received peritoneal injections showed areas of necrosis, interstitial adhesions, and calcified nodules in the liver, spleen and lymph nodes. The silicotic nodules produced their own collagenous stroma.

Experimental Group I

Group I animals were injected with the ash from a patient who died with bronchogenic carcinoma. The patient, H. G. S., age 53, had been a laborer in and around coal mines for many years. During the 2 years preceding his death, he had become short of breath and easily fatigued. He attributed his condition to the inhalation of dust and entered suit against the company that employed him. X-ray findings were all against silicosis and consisted of massive consolidation, more marked on the left side. At autopsy, the pathologic diagnoses were primary bronchogenic carcinoma of left lung; secondary carcinoma of pleura, pericardium, chest wall, liver, right lung and peribronchial lymph nodes.

Chemical Analysis. The left lung weighed 850 gm. and contained 0.83 mg. of silica per gm. of dried tissue. It was not further analyzed. The weight of the right lung was 940 gm. It contained 0.505 gm. of ash, of which 0.097 gm. were silica. The silica was all in the form of silicates; no free silica was found. As ordinarily expressed, there was 0.79 mg. of silica per gm. of dried tissue, or 0.079 per cent.

Three rabbits and three guinea-pigs were injected as described for control group III. All animals lived throughout the 23 months of the experiment. The rabbits were negative, and the only lesions found in the guinea-pigs which were injected intraperitoneally were collections of phagocytes and foreign body giant cells beneath the peritoneum, filled with brown pigment, and a few similar collections of cells in the spleen. There were no evidences of toxic action, no silica nodules and no fibrosis. The results in this group were typical of the "absorptive" reaction of Miller and Sayers.[11]

Experimental Group II

The second group of animals was injected with the ash of a case of silicotuberculosis.* The patient, J. H., age 70, had been employed for many years in a marble yard. The physical findings had been dullness at both bases, more marked on the right; vesicular sounds in the upper portions, and râles over the dull areas of both lungs. Pathologically, the gross examination of the lungs revealed dense, gray-black scarring of the upper portions of the lower lobes and lower portions of the upper lobes, many small, black, rubbery nodules throughout the remaining portions of the lungs and shaggy adhesions on the pleural surfaces. Sections contained many yarn-ball silicotic nodules, active tubercles and areas of dense fibrosis. Between the fibrous areas there were emphysematous spaces and air sacs filled with pigment phagocytes. Pathologic diagnoses were silicotuberculosis, emphysema, regeneration of pulmonary alveoli and chronic adhesive pleuritis.

Chemical Analysis. The weight of the two lungs, as received in formalin, was 1200 gm. They contained 6.210 gm. of acid-insoluble ash, of which 4.543 gm. were silica. If the right and left lungs be assigned 53 and 47 per cent respectively of the total lung tissue, then the silica per lung was 2.408 and 2.135 gm. respectively. Of this, 60 per cent was combined (silicates) and 40 per cent was free silica. Stated in the commonly used terms, the total silica amounted to 24.2 mg. per gm. of dried tissue, or 2.42 per cent, and the free silica was 9.7 mg. per gm., or 0.97 per cent.

Three rabbits were injected in the ear veins with 2 cc. of a 1 per cent suspension of ash on alternate days for 3 doses and with 2.5 cc. of a 1.25 per cent suspension for 4 doses. All rabbits lived for the entire 23 months of the experiment, when they were sacrificed. Grossly, there were hard nodules along the ear veins at the sites of injection and no other lesions. Microscopically, the changes were identical in the three animals and consisted of collections of dust cells in rosettes in the liver sinusoids and at the borders of the periportal tissues. There were similar, but somewhat larger, collections of dust cells in the splenic pulp and germ centers. The lungs were negative.

Three guinea-pigs were given 3 doses of 2 cc. each of 1 per cent suspensions at intervals of 2 days. One died before lesions appeared and 2 lived 23 months until the end of the experiment. In the gross, the latter both showed a few white, subperitoneal patches. Their livers and spleens contained many minute, grayish, pebbly spots, and their

* Contributed by Dr. Ivan Brown, Reading, Pa.

lungs presented small, blackish spots due to inhaled dust. Microscopically the subperitoneal nodules were made up of large collections of dust phagocytes situated in the fat. There was a feeble attempt at reticulation and encapsulation, and many giant cells. The liver and spleen showed collections of gray phagocytes in the sinusoids and in the pulp.

Summary of Group II Experiments. In spite of the rather high percentage of silicates and the presence of free silica, the results in all of the group II animals were rather weak examples of the absorptive reactions of Miller and Sayers.[11] Apparently the silicates or other components of the extract protected the tissue from the usual toxic effects of the free silica.

Experimental Group IV

The group IV experiments were made with lung ash of a case of silicotuberculosis and pneumonia. The patient, G. S., had been employed for many years in a coal mine as a motorman hauling coal cars in the mine. The tracks required constant sanding and the air he breathed was admittedly very dusty. He was a man beyond middle life who had suffered for many months from shortness of breath, fatigue and general poor health. His death was due to pneumonia and followed an acute illness of about 3 weeks. The lungs were brought in from a neighboring hospital.

The left lung weighed 775 gm. The pleural surfaces were covered with shaggy fibrous adhesions and in part with acute fibrinous exudate. The consistency was tough, fibrous and boggy. On section, there were many small, hard, gray-black nodules from 2 to 4 mm. in diameter and many large areas of fibrous thickening. In the upper, posterior portion of the left lower lobe there was a diffuse, acutely consolidated, portion. At the hilus there were several large, blackened lymph glands containing calcified gray nodules. Microscopically, some of the nodules presented the typical yarn-ball patterns of silicosis while others had caseous centers and were recognizable tubercles. The diffuse consolidated area was one of unresolved pneumonia with healing by organization and fibroblastic repair. Tuberculosis of the lymph nodes was proved microscopically.

The right lung weighed 2040 gm. The pleura was covered with a fresh fibrinous membrane. All three lobes were massively consolidated by lobar pneumonia. In addition to the pneumonia, the surface was studded with pinhead-sized silicotic nodules which were gray and rubbery. The pigment appeared much paler than in the left lung due to dilution with the acute pneumonic exudate. The hilus nodes resembled those on the left. Microscopically, all of the gross lesions were verified.

Incinerated sections from both lungs left an ash which was brownish gray, partly amorphous and party crystalline. Throughout all specimens there were abundant, highly refractive, heat-resistant metallic particles. Application of 1 per cent hydrochloric acid overnight removed the brown material and left the insoluble crystalline silica and amorphous silicates behind. The pathologic diagnoses were nodular silicosis, emphysema, pigment cell interstitial pneumonitis of both lungs, silicosis and tuberculosis of peribronchial nodes, bronchopneumonia, healed conglomerate tuberculosis and organizing pneumonia in the left lung, and lobar pneumonia with organization in the right lung.

Chemical Analysis. The left lung weighed 775 gm. and contained 2.968 gm. of acid-insoluble ash of which 1.375 gm. was silica. Of the latter, 72 per cent was in the form of silicates and 28 per cent was present as free silica. Expressed in the usual manner, there were 11.45 mg. of total silica per gm. of dried tissue, or 1.145 per cent. The corresponding figures for free silica were 3.2 mg. and 0.32 per cent.

The weight of the right lung was 2040 gm. It contained 3.276 gm. of ash and of this 1.611 gm., or 49.2 per cent, was silica. In the customary terms this was 5.7 mg. per gm. of dried tissue, or 0.57 per cent. The ratio of free to combined silica was not determined because previous experience had shown that significant differences were not to be expected from the samples of ash from a pair of lungs.

At once a discrepancy is seen in the silica of the right and left lungs when expressed as milligrams per gram of dried tissue, the left having almost exactly twice the concentration of the right. The explanation is found in the different amounts of acute exudate in the two lungs as indicated by the weights. On drying, the exudate yielded a residue similar in weight to that of normal tissue. The dry weight of the lung was thus much increased. The ash, on the contrary, undoubtedly represented material that had been inhaled before the onset of pneumonia and in quantities in proportion to the weights of the normal lungs.

If the weights of the normal lungs are taken as 360 and 410 gm. respectively, then the left lung is 47 per cent and the right is 53 per cent of the total tissue. The amounts of silica in the two lungs should be in the same ratio. Actually the ratio for the silica found in these lungs is 46:54. The weight of the right lung is about five times the normal. The figure for silica in milligrams per gram of dried tissue is therefore to be multiplied by five to indicate its true significance and becomes 28.5 mg. per gm., or 2.85 per cent. In the same way, the figure for silica of the left lung must be multiplied by 2.15, giving 24.6 mg. per gm., or 2.46 per cent. These are in satisfactory agreement. It is

from such considerations that we favor reporting silica per lung rather than per gram of dried tissue. In none of the lungs involved in this paper was the weight normal.

Rabbits. Three rabbits were injected in the ear veins on alternate days with 2 cc. of a 1 per cent suspension of the residue. One rabbit died during the ninth injection, 22 days after the first dose. Dust cells were found in groups in the liver sinusoids and in the venous spaces of the spleen. There was no fibrosis. Another rabbit received 8 doses in the ear vein and 4 doses intraperitoneally after the ear veins were closed. The animal died 6 months later of peritonitis and pneumonia. In the gross, the effects of silica were seen in large areas of necrosis and organizing abscesses in the abdominal walls. Microscopically, the silicotic nature of the abscesses was proved and incinerated sections showed much highly refractive crystalline material in the necrotic zones. The sections of the organs showed an extremely acute serous exudate with amyloid infiltration of the spleen and renal glomeruli. No amyloid could be demonstrated in the liver sinusoids, even with special stains. The only nodular silicotic lesions were along the ear veins and around the abdominal abscesses. The third rabbit received 9 doses in the ear vein and lived until the end of the experiment. Grossly, the ear veins had beaded nodules. The lungs had several blackened areas from air inhalation. The liver was in an advanced state of cirrhosis with multiple gray nodules and fibrous portions entirely devoid of hepatic tissue. The spleen was grossly negative. The lymph nodes were enlarged and partially replaced by gray nodules. Microscopically, the nodules of the ear veins, spleen and lymph nodes were made up of collections of phagocytes and their silica content was proved by incineration. The cirrhosis of the liver was extensive and the amount of periportal connective tissue greatly increased. There was proliferation or approximation of bile ducts and the fibrosed bands contained occasional nodules suggesting nodular silicosis.

Incineration proved the presence of silica particles in the cirrhotic areas although the number of particles was small in comparison to the extensive fibrosis.

Guinea-Pigs. All three of the original guinea-pigs in group IV, placed together in the same cage, were lost. As there was some of the suspension remaining, two other guinea-pigs were given single doses of 2.5 cc. intraperitoneally, and sacrificed at the end of 3 months. Grossly, they both showed yellowish white nodules at the sites of injection and in the omentum and lesser peritoneum. No lesions were found in the lungs. The Kupffer cells of the livers showed slight proliferation. There were early nodular collections in the spleens and lymph nodes. The

peritoneal lesions had the appearance of early progressive silicotic nodules.

Summary of Group IV Experiments. The only rabbit injected intravenously which lived throughout the experiment developed cirrhosis of the liver, and all of the animals injected intraperitoneally produced nodules of the progressive type described by Miller and Sayers.[11]

Experimental Group V

The experiments in group V were made with suspensions of lung ash from a case of massive silicosis in which there were a few recognizable healed tubercles. The patient, G. H., age 35, colored, had been employed as a castings cleaner for 11 years in a rolling mill and foundry. For 2 years he had had a cough, had become easily fatigued, and had lost weight. For several months he had been bed-ridden and under treatment for "pulmonary tuberculosis."

Left Lung. The pleura was thickened and covered with old fibrous adhesions. On section the lung was dark, slaty red and the consistency was resilient. The posterior, upper and central portions of the upper and lower lobes were solid and air-free. The lower anterior margin of the upper lobe and about one-fourth of the lower lobe contained air. The color of the air-containing portions was red-brown in contrast to the slaty red-gray of the solid parts. There were no cavities. The peribronchial nodes were enlarged and filled with black pigment and white granular nodules.

Right Lung. The pleura was covered with adhesions. All three lobes were consolidated and air-free except the margins of the basal portions. The appearance was essentially the same as in the left lung. Microscopically, no air sacs or alveolar walls remained in the solid portions. Cellular areas containing masses of phagocytes alternated with irregular islands of collagenous, practically nucleus-free, connective tissue. The collagenous zones were nonvascular. In the mucosa of the bronchioles there were occasional miliary tubercles. Large sections, almost an inch square, were taken in which no air sacs were present. In the peribronchial nodes there were numerous typical yarn-ball silicotic nodules, with no active tubercles.

Incineration showed much particulate crystalline matter between the collagenous masses with fewer particles in the collagenous masses themselves. Pathologic diagnoses were advanced massive silicosis, active peribronchial miliary tuberculosis, chronic adhesive pleuritis.

Chemical Analysis. The left lung weighed 1025 gm. It contained 5.680 gm. of ash and of this 4.266 gm., or 75.1 per cent, were total silica. This latter was made up of 73 per cent of free silica and 27 per

cent of combined or silicate silica. As ordinarily reported there were 26.8 mg. of total silica per gm. of dried tissue, or 2.68 per cent. The free silica was 19.5 mg. per gm., or 1.95 per cent.

The right lung weighed 1100 gm. and contained 5.959 gm. of acid-insoluble ash of which 5.002 gm. were total silica. This was made up of 63 per cent of free silica and 37 per cent of combined (silicate) silica. In the dried tissue there were 29.7 mg. per gm., or 2.97 per cent, of total silica and 18.8 mg. per gm., or 1.88 per cent, of free silica. These figures are again deceptively low because of exudate in the lung. Free silica and ash were higher in these lungs than in any others in our series. Injection experiments were made as in the previous groups.

Rabbits. One rabbit received 9 doses of 2 cc. each of a 1 per cent suspension of the extract and died on the 47th day with pneumonia. The organs and surfaces were negative save for silica beading along the ear veins. Microscopically, there were small silica tubercles in the germ centers of the spleen and nests of silica phagocytes in the periportal areas of the liver. The periportal connective tissues were slightly increased in amount. The second rabbit died 6 months after injection. Grossly, the ears were thickened and fibrous, there was a pronounced cirrhosis of the liver and the spleen was enlarged and roughened. Microscopically, the perivascular lymphoid tissue was absent and replaced by fibrous thickening of the adventitia. Occasionally the adventitial thickenings formed small nodules with a few enclosed phagocytes filled with crystalline particles. The spleen contained many silicotic tubercles. Some were in the germ centers and some were in the pulp. Many of them showed a newly formed fibrous stroma. The liver showed advanced cirrhosis which was identical microscopically with that found in the silica controls. There was much fibrous replacement by wide bands and a few collections of phagocytes filled with crystalline particles in the bands. The bile ducts were compressed and apparently isolated in the newly formed connective tissue. The kidneys showed diffuse interstitial fibrous hyperplasia with tubular distortion and compression. The glomeruli did not seem to be affected. The remaining rabbit died during the fourth injection.

Guinea-Pigs. The guinea-pigs in this series each received 3 intraperitoneal doses of 2.5 cc. of a 2 per cent suspension of the lung extract. In one the pleura was mottled, the spleen was enlarged and showed nodules on the capsule. The liver was filled with miliary nodules and had a calcified plaque beneath the capsule. The diaphragm and parietal peritoneum were covered with white nodules. The intestines were adherent to the parietal wall and there were soft, partially calcified, granulomatous masses in the adhesions. The omentum was rolled up

and small, gritty, silicotic abscess foci were distributed along the lower border of the stomach. The intestinal loops were adherent to each other and the mesenteric nodes were enlarged and contained white spots. Microscopically, the lungs contained some well developed perivascular silicotic nodules which replaced the areas usually occupied by lymphoid tissues. The nodules were sometimes larger than the vessels. The liver showed fibrosis in some places and collections of dust cells with giant cells in others. The nodules produced their own collagenous stroma. The spleen was filled with compact silicotic nodules. The granulomas of the parietal wall and between the intestinal loops were typical of those produced in the controls with pure silica. The lymph nodes were replaced by masses of phagocytes with areas of necrosis, and several minute collagenous nodules were present. The adventitia of the vessels of the adhesions were greatly thickened and sometimes necrotic. In places the media was encroached upon and a type of medial sclerosis produced. The findings in the second guinea-pig were practically the same as those of the first. The third guinea-pig died too early in the experiment to show changes.

Summary of Group V Experiments. Of the six animals in the experiment only one rabbit and two guinea-pigs lived for 6 months or more. In all of these, extensive silicotic lesions identical with those in the silicotic control group were produced.

Summary and Discussion

Experiments were conducted by injecting into rabbits and guinea-pigs chemical ash from four sets of lungs sent to the laboratory for analysis and the results controlled with a series injected with suspensions of pure silica. The ash was selected as the representative substance because it contained silica and silicates in approximately the same proportion as they were recovered from the lungs. Suspensions of similar concentration and similar quantities of the respective ashes were used in all injections. The animals in the control group presented the typical lesions described by Gye and Purdy,[9] Miller and Sayers,[11] Gardner and Cummings,[10] and others, and included nodules of the lungs, spleen, lymph nodes, peritoneum and blood vessel walls. In one rabbit injected intravenously, which lived for the 23 months of the experiment, there was a well developed cirrhosis of the liver. Of the group I series, which received lung ash from a case of bronchogenic carcinoma in which 0.079 per cent of the ash was total silica without free silica, all lived throughout the experiment and presented mild lesions of the type designated by Miller and Sayers as the "absorptive type." Group II animals received injections of lung ash from a case

of silicotuberculosis in a marble worker that contained 2.42 per cent total silica and 0.97 per cent free silica. Five of the six animals lived throughout the experiment and all showed pronounced absorptive lesions without fibrosis. The results in this series were not as anticipated and indicated that the silicates and other contaminating dusts protected the animals from the toxic action of the silica. In group IV the animals were injected with suspensions of lung ash from a case of silicotuberculosis that contained 1.145 per cent total silica, of which 0.32 per cent was free silica. Progressive granulomatous lesions were obtained in the peritoneum, lymph nodes and spleens. The only rabbit that lived for the duration of the experiment developed cirrhosis of the liver comparable to that found in the silica control. The animals of group V were inoculated with suspensions of ash from a case of massive silicosis in which the ash contained 2.68 per cent total silicates and 1.95 per cent free silica. One rabbit and two guinea-pigs lived 6 months or more and in all typical silicotic nodules of the lungs, liver, spleen and lymph nodes were produced. The rabbit presented advanced cirrhosis of the liver and the guinea-pigs had calcified granulomas of the peritoneum.

Three of the findings require additional discussion; *viz.*, cirrhosis of the liver, nodules of the lungs of guinea-pigs which received only intraperitoneal injections, and adventitial lesions in the walls of vessels associated with granulomas.

Gye and Purdy [9] described cirrhosis due to repeated sublethal injections of colloidal silica and Gardner and Cummings [10] used cirrhosis as a positive criterion in testing unknown substances for silica content. It was to be expected that extensive cirrhosis would be found in the silica control group and in the group V animals in which 73 per cent of the ash was free silica. It was more surprising to find cirrhosis very pronounced in the group IV rabbit where the ash contained only 28 per cent free silica. Incinerated sections showed relatively few crystals in the fibrous areas. Fifteen rabbits were used in the combined experiments and in only three instances was cirrhosis found. These were in the silica control group III and in test groups IV and V. Since other rabbits that lived 6 months or more showed silicotic liver nodules but no cirrhosis, the probability of an extraneous cause was not great.

The guinea-pigs injected intraperitoneally with pure silica and with the suspension of ash from the case of massive silicosis showed in the lungs well developed perivascular nodules composed of agglomerations of silica phagocytes. In explanation of these findings the work of Ungar and Wilson [12] is of interest. They produced chemical peritonitis in one set of animals, marked the phagocytes of the exudate *in vitro*,

washed the cells and reinjected them into a second series. They found that regardless of the site of injection (some injections were made directly into the portal vein) the marked phagocytes concentrated in the lungs. It seems likely that the silica in suspension, injected into the peritoneal cavity, taken up locally and found regularly in the livers and spleens, also reached the lungs in sufficient amount to produce the perivascular nodules.

Some of the perivascular lesions in the margins of silicotic granulomas of the peritoneum were most striking, while the vessels of the same animal taken at a distance from the granulomas appeared normal. The affected vessels had very thick walls. The intimal coats were normal. The muscular coats were thickened unevenly owing to irregular replacements of the muscle cells by a waxy type of connective tissue. The adventitial coats were very wide and fused with the stroma of the granulomas. They too presented a myxomatous appearance and were generally infiltrated with phagocytes having one or more nuclei. Both arteries and veins were affected. The vascular changes were comparable to many of those seen in advanced silicosis of human lungs.

Conclusions

1. Experimental silicotic lesions were produced in animals with injections of suspensions of ash recovered chemically from the lungs of cases of human silicosis.

2. The lung extracts were made from whole lungs and care was used to prevent overheating and overacidifying so that the silica was recovered in the same condition, as nearly as possible, as it existed in the lungs. Silica extracted in this way had not lost its toxic activity during its sojourn in bodily tissues.

3. Just as had been found in human pneumoconiosis, the activity of the ash appeared to depend upon the amount of free silica present and upon the modifying influences of accompanying substances in the residue.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

Plate 20

Fig. 1. Collection of phagocytes filled with fine silica particles replacing a perivascular lymph node in the lung of the rabbit in the control group. Necropsy performed 23 months after intravenous injections with a 1 per cent suspension of pure silica. × 130.

Fig. 2. A more fibrotic silicotic nodule from the same animal as in Figure 1. The adventitia of a large vein is involved in the fibrotic process. In the left upper corner there is a collection of phagocytes loosely arranged in the same manner as that of the nodule seen in Figure 1. × 130.

Fig. 3. A fibrous nodule of late type in the lung of a rabbit of group IV, injected intravenously with the ash from a human lung. The nodule is at the site of former perivascular lymphoid tissue. × 130.

Fig. 4. Nodular silicotic phagocytes in the perivascular lymphoid tissue of the lung of a guinea-pig of group V, injected intraperitoneally 23 months previously with the ash of human lung with massive silicosis. × 130.

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PLATE 21

FIG. 5. Collection of silica and carbon phagocytes in the perivascular lymphoid tissue of the lung of a rabbit injected intravenously with ash of human lung (group IV). × 130.

FIG. 6. Collections of silica phagocytes in the periportal connective tissue of a rabbit injected intravenously with the ash from human lung (group II). Throughout the livers of the rabbits in this series there were similar collections of silica phagocytes in the liver sinusoids. × 130.

FIG. 7. Collection of silica phagocytes in the spleen of the same rabbit as shown in Figure 6. × 130.

FIG. 8. Perivascular collections of silica phagocytes in the spleen of a rabbit injected intravenously with a suspension of the ash from human lung (group V). Some of the nodular collections occupy germ centers and some are diffusely distributed throughout the pulp. × 130.

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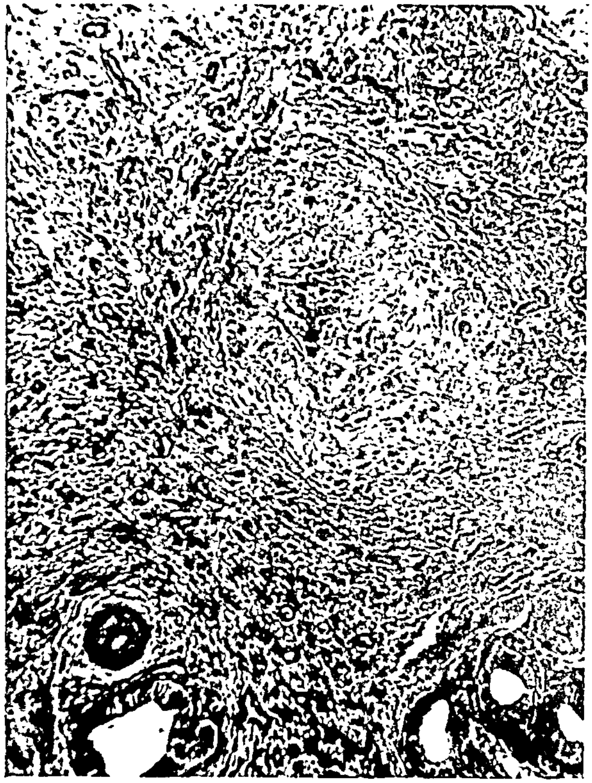
PLATE 22

FIG. 9. Low-power view showing cirrhosis of the liver of a rabbit in the control group which had been injected 23 months previously with a 1 per cent suspension of pure silica. × 65.

FIG. 10. Higher power view of the same liver as in Figure 9 to show fibrotic nodule occupying a cirrhotic area. × 130.

FIG. 11. Cirrhosis of the liver of a rabbit injected 6 months previously with a 1 per cent suspension of ash from human lung (group V). × 65.

FIG. 12. Cirrhosis of the liver from a rabbit injected 23 months previously with a 1 per cent suspension of ash from human lung (group IV). × 65.



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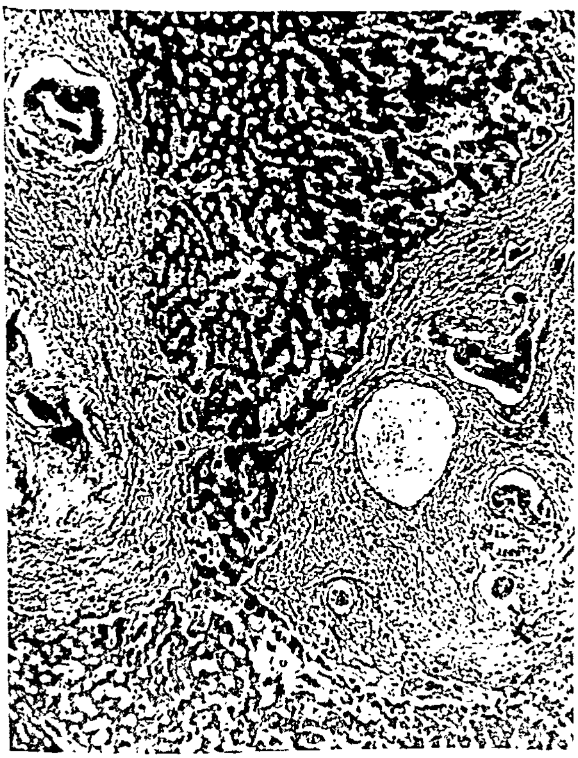

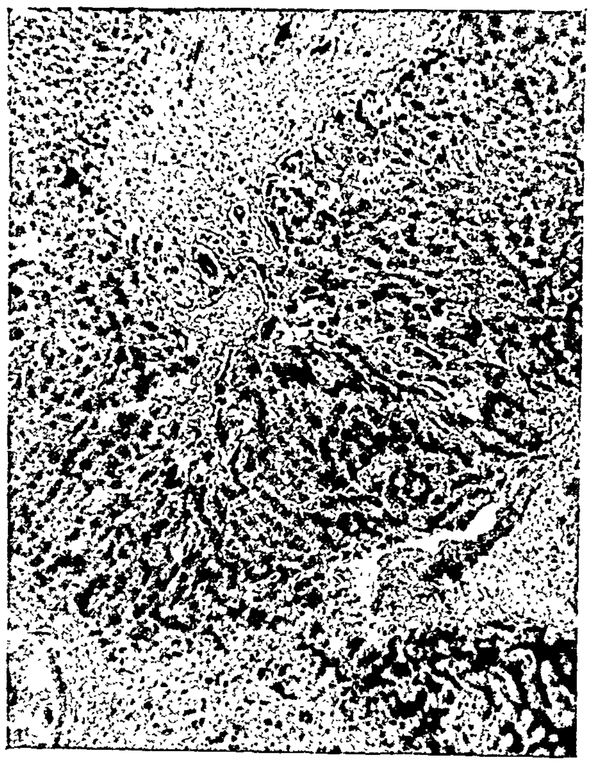

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Haythorn and Taylor

Experimental Silicosis

INFLUENCE OF AGE ON THE GROWTH OF LYMPHOMAS *

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More rapid growth of tumors is thought to occur in young than in old persons. Even though this idea is accepted clinically, an experimental clarification is warranted.

A feasible method of attacking this problem would be to compare the growth of transplantable tumors in animals of different age groups. Of such tumors, transplantable lymphomas of laboratory mice and rats were found to be satisfactory. When inbred strains of animals are used, transplants of lymphomas take in 90 to 95 per cent of trials and grow rapidly, a fact which makes data easy to obtain. The tumors are readily minced; and without dilution, by using a 1 ml. syringe, a known dose of cells can be administered to each animal. Because of the rarity of necrosis, the amount of living tumor may be fairly accurately measured and weighed.

The concept of "more rapid growth" of a tumor in the young has probably been most closely connected with the highly fatal tumors of childhood. It has also been considered that less malignant tumors increase more rapidly in size in young persons. For these reasons it was decided to study the following characteristics of lymphomas in animals of different age groups: (1) tumor growth by daily measurements and, in some cases, final weight of the tumor at the time of death; (2) changes in weight of tumor-bearing animal; (3) weight of tumor grown, as compared with the weight of the animal; and (4) mortality as measured by percentage of survivals. Microscopic studies were also made and mitotic indices computed. All comparisons of weights of animals were adequately controlled with litter-mates.

The young adult, and the old animals were fed on a standard Purina dog chow diet. The very young animals were unweaned at the beginning of each experiment; and although they were weaned during the experiment, this did not visibly affect the tumor growth.

Materials

Four lymphomas were used in this study. Three, when inoculated subcutaneously, remained localized until the disease was in its end-stage before they became generalized. These were a malignant thymoma (lymphoblastic type), lymphoma A of strain A mice, and a lymphosarcoma-leukemia of rats (Murphy). These three were used

* Received for publication, April 3, 1944.
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for study of the growth of the local tumor. The fourth, a generalized lympholeukemia, Y103, which results in a rapidly fatal generalized disease, without formation of large tumors, was used to study mortality rate. All of these tumors had been carried in transplant for a number of generations, and, at the time they were used, their growth had become constant within each strain.

Malignant Thymoma

On May 13, 1942, a female mouse, 7 months old (C57 Black × C3H), was found in great respiratory difficulty. The animal expired shortly and upon necropsy showed a large, conical mass occupying the site of the thymus and molded into the shape of, and filling, the whole upper thorax. It was attached to the posterior aspect of the sternum and had invaded the upper mediastinal structures. The tumor measured 15 by 10 by 6 mm. The cause of death was mediastinal compression. There was no general glandular enlargement, and careful gross examination did not reveal any other abnormalities. The diagnosis, on frozen section, was malignant thymoma of the lymphomatous type (lymphoma invading surrounding mediastinal structures). An immediate subcutaneous transplant to the groin was made into five C57 Black × C3H animals.* Sections from the tumor showed it to be made up of large, regular, round or oval cells with large nuclei and scarce cytoplasm, the cells resembling lymphoblasts. One to seven mitoses were found per oil-immersion field. The cells were arranged in no particular fashion and had destroyed normal thymic structures. No areas of necrosis were seen, and no tissues beyond those directly affected showed significant changes except for the bone marrow, which was extremely hyperplastic and possibly had early involvement. In transplantation the tumor grew at the site of inoculation to enormous proportions, 30 by 28 by 15 mm., or larger (Fig. 1), and late in the disease general blood stream involvement took place. One such animal showed a total white cell count of 87,000 per cmm. with 90 per cent lymphocytes and lymphoblasts. Almost every organ in the body showed neoplastic cells, which were found as a moderately heavy, dispersed infiltration in the liver and were most abundant in the lymphoid tissue and bone marrow. The microscopic pattern of the tumor is shown in Figure 2.

At the time of the transplantation for the growth studies, the tumor already had been passed through 4 generations of transplants. At this

* On May 28, 1942, an almost identical tumor occurred in a male mouse, 10 months old, of the same derivation, which had received a stilbestrol pellet subcutaneously some months previously. This later tumor had already escaped from its site of origin and had metastasized to the spleen and the liver.

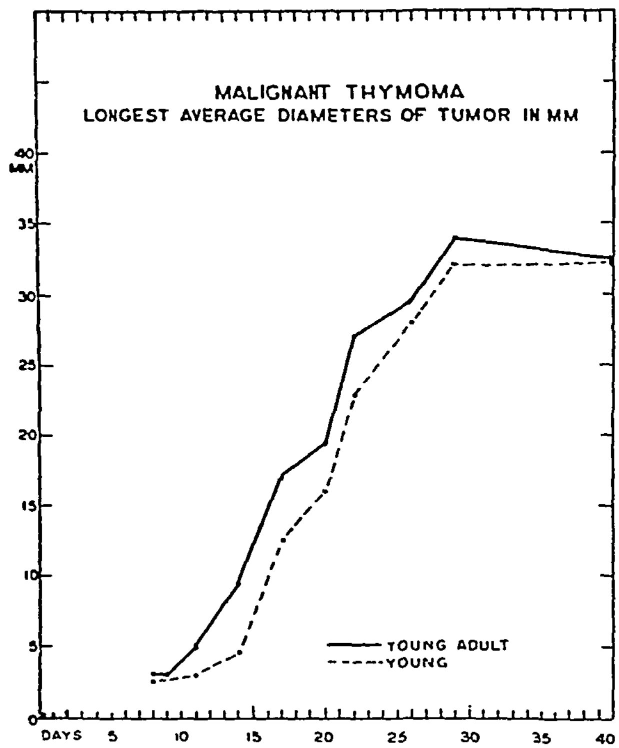
time it ordinarily killed between the sixth and eighth week after transplantation. The average size and weight of the tumors is given in Table I. On the morning of the experiment, an animal bearing a

TABLE I

Average Weight of Malignant Thymoma in Very Young and in Older Mice

Age (in days)	Number of mice	Average initial weight	Average weight (with tumor) at death	Weight range	Average weight of tumor at death
		(gm.)	(gm.)	(gm.)	(gm.)
12	21	4.8	22.4	1.4	8.0
42	34	17.1	31.0	6.0	12.3

thymoma which had grown for 4 weeks was killed, the tumor minced under aseptic conditions, and a dose of 0.05 ml. of minced tumor was injected subcutaneously into the right groin of each animal. The

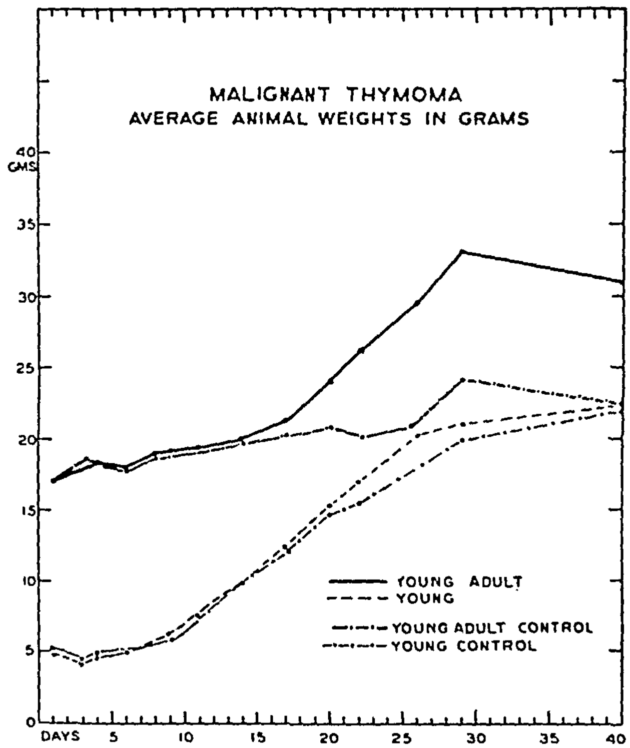


Text-Fig. 1. Longest average diameters of transplanted malignant thymomas measured by vernier caliper. The growths in the two age groups parallel each other.

tumors became palpable the next day, and their final masses in each age group were nearly identical. The average tumor sizes are shown in Text-Figure 1.

There was surprisingly little difference between the two age groups in tumor size, even though the initial weight of the very young animals was approximately only one-fourth that of the older ones. The gains in

body weight of the normal control animals as plotted were less than those of the tumor-bearing animals. The levelling off phase is difficult to understand except on the basis of general reduction in rate of body growth in both groups. Text-Figures 1 and 2 suggest two distinct phases in the growth of this tumor: an early rapid growth, and a late phase in which the growth drops off markedly. The groups were intact at the end of the experiments. Text-Figure 2 shows that the deceleration of the young animals in this late phase was not so extensive as that of the older ones. At first this phase of retardation was considered

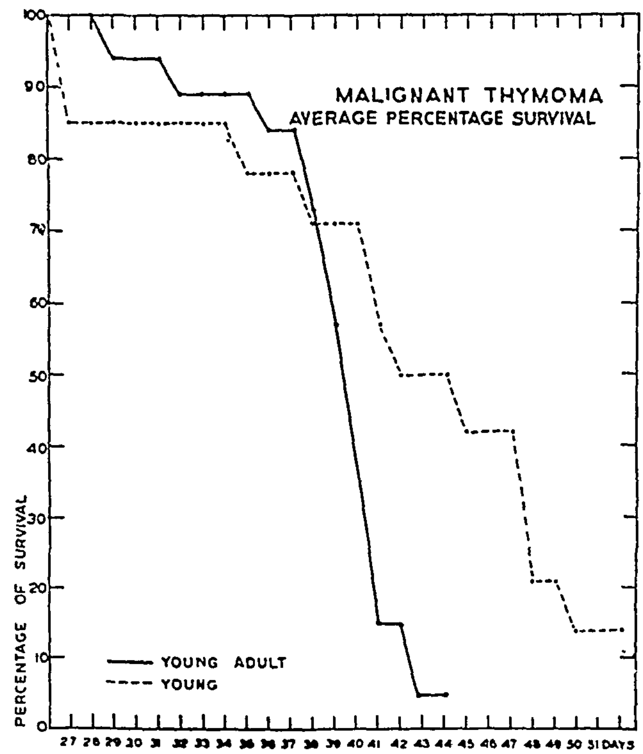


Text-Fig. 2. Average daily, or alternate day, weights of tumor-bearing animals after tumor inoculation and of noninoculated controls.

to be the lethal phase of the tumor until it was discovered that the weights of the control animals were showing a parallel retardation. From this, it appeared that the rate of tumor growth was somewhat dependent upon general body growth. When killed, the control animals in the young groups weighed approximately the same as their tumor-bearing litter-mates, and yet almost half of the total weight was tumor. In the group of mice 42 days old when implanted, the average tumor-bearing animal at death weighed 7.5 gm. more than the litter-mate control.

From the data it seems plausible to conclude that during the phase of rapid growth the tumor-bearing animal grows the same amount of

tissue, even though the tissue is neoplastic, as the litter-mate grows of normal tissue—and that the growth of neoplastic tissue must be at the expense of other tissues. In somewhat older animals, as long as there was no general effect upon the body, the tumor-bearing animal actually grew more tissue than did the litter-mate control, *i.e.*, the neoplastic tissue grew under the same general nutritional conditions as normal body tissues although more rapidly. In both age groups the average weight of the animal minus the tumor was less than that of the normal control. Although almost all of the animals were near



Text-Fig. 3. Survival in the different age groups.

the fatal limit of the tumor at the termination of the experiment, little general effect upon the body was noted. In this experiment the number of malignant cells injected into each animal of each age group was approximately the same, and the final mass of neoplastic tissue grown was greater in the older animals (Table I). Percentage survival curves showed that the younger age group lived longer (Text-Figure 3).

Lymphoma A

The next tumor to be studied was another lymphoma that arose spontaneously in a strain A mouse. It was discovered on May 9, 1940, in a male mouse, 4½ months of age. At that time the animal had generalized lymph node enlargement and a total leukocyte count of

92,000. The differential count showed 76 per cent lymphocytes and 23 per cent oxidase-negative blast forms. The post-mortem examination showed large mesenteric nodes (20 by 8 by 8 mm.), and cervical, axillary and inguinal nodes which averaged 10 by 8 mm. All of them were soft and gray-white with no gross necrosis. The thymus escaped. The spleen was purple-gray and tense, and measured 25 by 15 by 5 mm. Both liver and kidneys were gray and slightly enlarged. Histologic examination showed that the lymph nodes were filled with diffusely growing, round, basophilic cells with large nuclei, which had replaced the normal tissue. The same cells formed typical leukemic deposits in the liver, spleen, kidneys and bone marrow. There were many mitotic figures.

The diagnosis of leukemic lymphomatosis was made and the tumor

TABLE II

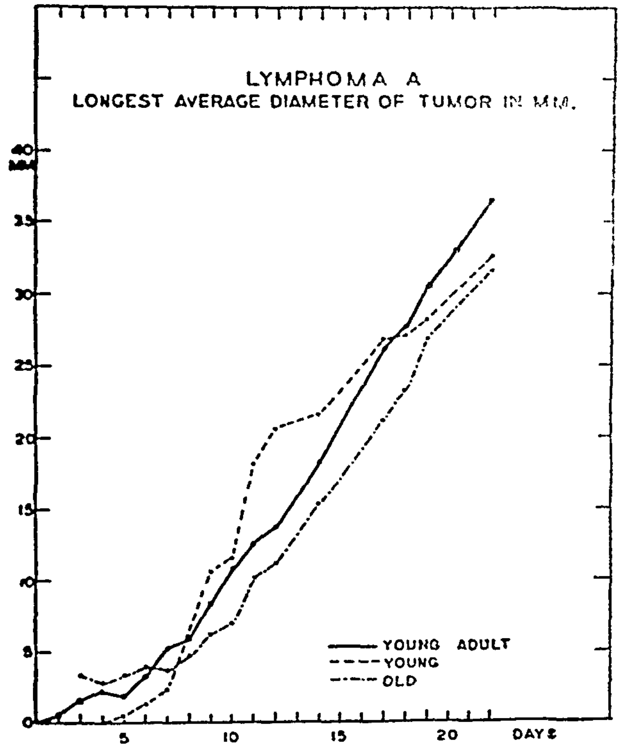
Average Weight of Lymphoma A, and Average Weight of Mice in the Three Age Groups

Age group	Number of animals	Average initial weight	Average weight at death	Average weight of tumor at death
		(*gm.*)	(*gm.*)	(*gm.*)
Unweaned (12 days)	18	6.9	21.3	13.0
4–5 months	17	28.3	37.6	13.8
13 months	20	27.8	32.5	9.8

then transplanted. At the time of the present experiment it had been transplanted nineteen times. The original sections were similar to those in the present transplants (Figs. 3 and 4). Sixty animals were used in the present experiment, 20 to each group. Except for dosage, the technic was similar to that used for the thymoma. The tumors of origin were minced together, and 0.05 gm. per 10 gm. of body weight was injected rapidly into the subcutaneous area of the groin. The dose was given in the proportion of a milligram of neoplastic tissue per gram of body weight to determine whether the amount of tumor injected would grow to tumors of varied size. Not more than 30 minutes was allowed to elapse between the time the animal bearing the original tumor was killed and the last animal received its tumor injection. Three age groups were used in this study. Although the dose of tumor inoculum was proportional to the weight of the animal, this made little difference since even the smallest dose of tumor cells produced neoplasms. Table II gives the data on the comparative weights in this series, and Text-Figure 4 a comparison of the longest average diameters of the tumors.

Grossly, there was surprisingly little difference in the way this tumor

grew in animals of each age group. Regardless of the size of the animal's body, the tumors in the oldest group weighed less than those in the other two groups. The animals were all killed on the 22nd day of the experiment. The mitotic index of the tumor was 4.7 in the young animals, 5.8 in the middle-aged and 4.8 in the old animals.* In this tumor the transplants appeared almost simultaneously in each



Text-Fig. 4. Longest average tumor diameter in millimeters for the three age groups bearing lymphoma A. No significant difference could be determined between the different groups at the time the animals were killed.

age group; a few of the oldest animals showed tumor before either the young or the middle-aged.

Lymphosarcoma-Leukemia (Murphy)

The third tumor studied was the rat lymphosarcoma-leukemia isolated by Murphy[1] in 1939. Although this tumor arose in an animal which received dibenzanthracene, it may possibly be spontaneous in origin. Its characteristics are similar to those of the other lymphomas. When the tumor was received, it had already been through a number of passages. I had carried it through more than 10 passages before

* The mitotic indices were determined by averaging the number of mitoses seen by multiple focusing in ten oil-immersion fields (× 900). In this experiment, the periphery of the tumor was chosen for mitotic counts; areas with necrosis, other tissues, or gross tissue spaces were not included.

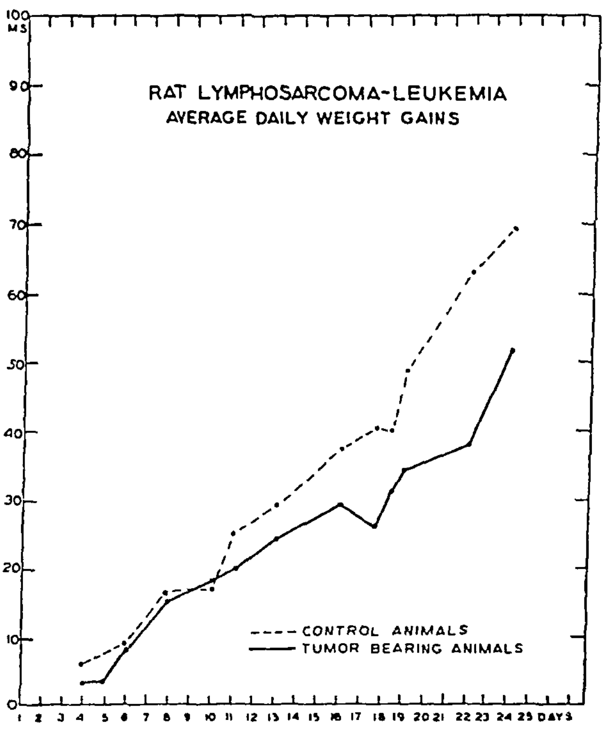
it was used in the present experiment. The percentage of takes in 102 animals inoculated with the tumor was 93. The tumors grew rapidly in the subcutaneous area of transplantation as large, soft, gray or gray-yellow masses with little or no necrosis (Fig. 5). Subcutaneous transplants killed the animals in an average of 12.6 days; a few animals survived as long as 25 days. Late in the disease there might be extension to the regional nodes. The drawing of a high-power field (Fig. 6) shows that the tumor is made up of large cells of lymphoblastic type.

The experiments with this tumor were made in a series of rats whose ages ranged from young, unweaned animals to young adults, and whose weights varied from 15.0 to 124.0 gm. Table III gives the data on the average weights, and Text-Figure 5 shows the contrast between tumor-bearing animals and controls. This suggests that the tumor may have

TABLE III

Average Weights of Unweaned and Young Adult Rats Bearing Lymphosarcoma-Leukemia (Murphy)

Age group	Number of rats	Average initial weight	Average weight with tumor at death
		(gm.)	*(gm.)*
Unweaned	18	20.5	35.5
Young adult	23	112.5	157.0

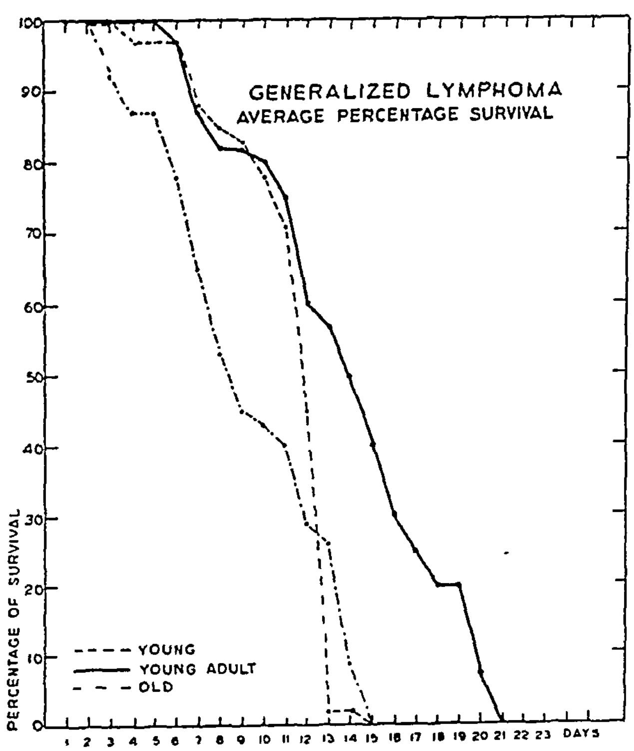


Text-Fig. 5. Weight gains of controls and of rats bearing rat lymphosarcoma (Murphy).

an early general effect, resulting in cachexia to the animals carrying it. Since few of the animals bearing the neoplasm survived beyond the second week, the curves for the last 10 days are not to be considered as absolutely accurate.

LYMPHOMA-LEUKEMIA Y103

Lymphoma-leukemia Y103, a generalized leukemia of dba strain mice, was used in the study of the mortality rate in three different age groups. This tumor had been carried through many generations. Upon subcutaneous inoculation into the flank of an animal, generalized disease sets in very rapidly. There is general body dissemination, almost every organ becoming involved. The mesenteric lymph nodes become enlarged, the spleen is large and tense, and the axillary, inguinal and submaxillary nodes may also become involved. Microscopically, the neoplasm appears to be made up of large cells with large nuclei and scant cytoplasm. Histologically, this neoplasm resembles very closely the other tumors studied. The experiments on growth were carried on in two series of animals, 62 in the first, and 71 in the second. The animals of the first series were injected with 0.025 ml. per 10 gm. of body weight, and those in the second series received a total dosage of 0.05 ml. The results are summarized in Text-Figure 6 and Table IV.



Text-Fig. 6. Average percentage survival rates of animals bearing generalized lymphoma-leukemia Y103.

The mitotic indices were obtained as were those for lymphoma A, except that mesenteric nodes were used as the tissue from which to obtain counts. Typical fields were chosen. The involvement of the mesenteric node is constant in this disease, and no normal lymphocytes were included in the microscopic pictures. The mitotic indices were: young, 3.2; young adult, 3.4; and old, 3.5. As nearly as could be determined, age made no difference in the rate of tumor growth. A few older animals, however, died very quickly after the tumor was injected.

TABLE IV

Average Time of Death of Animals Inoculated with Tumor Which Produces a Generalized Lymphoma

Age group	Number of animals	Average time of death
		(*days*)
Unweaned	45	10.4
4–5 months	47	13.0
14 months	46	8.7

DISCUSSION

It is difficult to give an exact interpretation of the clinical rule that malignant neoplastic disease proceeds most rapidly in youth. Does this mean that a tumor in a child grows faster, is more difficult to eradicate, or kills more quickly than in an adult? The embryonal nature of many tumors in children (retinal glioma, adenosarcoma of the kidney, neuroblastoma of the adrenal, and teratoma) may endow them with a higher intrinsic growth rate than tumors in adults. The prognosis with malignant neoplasms in childhood is usually considered grave.[2] This, however, may be related to late diagnosis and the inapplicability of diagnostic and therapeutic procedures used in adults. The clinical manifestations are usually advanced when the patient is first seen.

The only fair comparison which could be made would be between tumors of the same kind in children and adults, and tumors known not to be under an endocrine influence. Clinically, when this comparison is made, it is rather surprising that the outcome, as measured by end results, is often about the same for each age group. The end results of treatment of teratoma in children are about the same as those in adults.[3] The only comparison that has been made on adenosarcoma of the kidney shows that prognosis is as favorable in children as in adults. Carcinoma of the breast in a large series showed no significant differential survival rate in age groups from 31 to 80 years.[4] Melanomas of infancy and childhood are of low-grade malignancy and seldom metas-

tasize,[5] in distinct contrast to the melanotic neoplasms of adults. There may be an endocrine influence in this malignancy. Reports are varied with regard to leukemia, which is usually of the acute type and rapidly fatal in children.[6] Hodgkin's disease in children is well known for its better prognosis. The prognosis of cancer of the reproductive organs in female children is extremely unfavorable.[7] It is difficult to escape the idea that carcinoma in the child tends to pursue a more rapid clinical course than in the adult. This may be true clinically because vital zones are of much smaller dimensions in the child and a tumor growing at the same rate in young and old would consequently kill sooner in the young. Since the tumors in the present experiments were subcutaneous, this factor was largely ruled out.

It is of importance to discover whether the rate of tumor growth actually parallels the rate of body growth. In young animals this would appear to be the case prior to general bodily effect of the disease. On the other hand, the same or greater amounts of neoplastic tissue were found in young adults and older animals which gained or lost but a few grams in weight during the experiment. This would suggest that tumor growth is not related to the anabolism of youth beyond the nutritional factors involved. As a matter of fact, it is amazing that the relatively large tumors were able to receive sufficient nourishment in small animals as easily as in larger animals. Although the experiments reported herein were short-termed, the rate of tumor growth could be contrasted in young animals that were rapidly gaining weight, young adult animals in which the growth rate was slower, and older animals in which it was relatively stationary. The one factor of growth of the animal may thus be compared with the growth of the tumor. The experiments are useful because a tumor of constant growth rate was used and was placed in varying age environments, yet it could not be shown that age influenced either the rate of tumor growth or mortality. It appears, therefore, that the property of growth in these transplantable tumors is probably completely dissociated from that of general body growth except for nutritional requirements. We may assume, up to the point of general bodily debility, that the nutritional requirements in these experiments were adequate.

The mortality curves and rates in the age groups were not essentially different. In a number of old animals whose deaths occurred within 3 to 4 days after tumor inoculation, microscopic examination of the tissues showed that death was caused by neoplastic infiltration. This fact suggests that the debilitated state of the old animals renders them more susceptible to the fatal outcome of tumor growth.

The experiments might be criticized because variable doses of tumor

inocula were used, if it were not for the constancy of results and the large groups of animals used. A dose relative to body weight—so many milligrams of tumor to so many grams of body weight—was tried, then a standard dose was used in all age groups regardless of weight. In both, sufficient cells were used to produce tumor takes, but no variation in rate of tumor growth with dose could be found.

In at least one tumor, the malignant thymoma, it was possible to show that the rate of tumor growth actually paralleled the rate at which normal control animals were laying down tissue. In a way, this would characterize neoplastic tissue. Even though a smaller animal was not able to deliver the same amount of nutrition to the tumor area, nonetheless, the tumor utilized the necessary nutritional elements to show unrestrained growth. This did not restrain growth, regardless of the animal's age. The unrestrained growth of tumors fits in well with the known embryonic-histologic and metabolic characters of tumor cells.

Since it was not possible to correlate tumor growth with normal whole body growth (age), it seemed plausible to try to relate tumor growth to the regrowth of tissues in healing wounds. Some experiments indicate that healing in the young is more rapid because it begins earlier and is not caused by an actual increase in rate of growth.[8] If the growth phases are then compared, the factors, except for nutrition, that control the rate of growth are different from those that control the rate of wound healing. Perhaps the laws which govern wound growth are very closely related to those of normal tissue growth, whereas those of neoplastic tissue are far removed. Mitotic indices lend support to Ewing's[9] statement that behavior of a neoplasm is determined mainly by the "properties" of the tissue of origin, *i.e.*, by its original histologic-growth characteristics.

Summary

Four transplantable lymphomas were studied in animals of different ages. With regard to local lymphomas, the gain in weight during tumor growth was accounted for in the young animals by gain in the normal tissues (one-half), and also in the tumor. In older animals the gain in weight could be accounted for only by the amount of neoplastic tissue grown (the animal's tissues lost weight). In one instance the average tumor weight of the older age group was greater, in another instance slightly less, than that of the younger age group.

It was demonstrated with the malignant thymoma that these neoplasms increased in mass one-half as rapidly in young animals as did normal young growing tissues. All of the local tumors differed

from normal tissues in their ability to continue this growth unchanged beyond the slowing down of normal tissues.

No significant difference in mortality could be demonstrated in animals of different age groups when inoculated with a generalized lymphoma.

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[*Illustrations follow*]

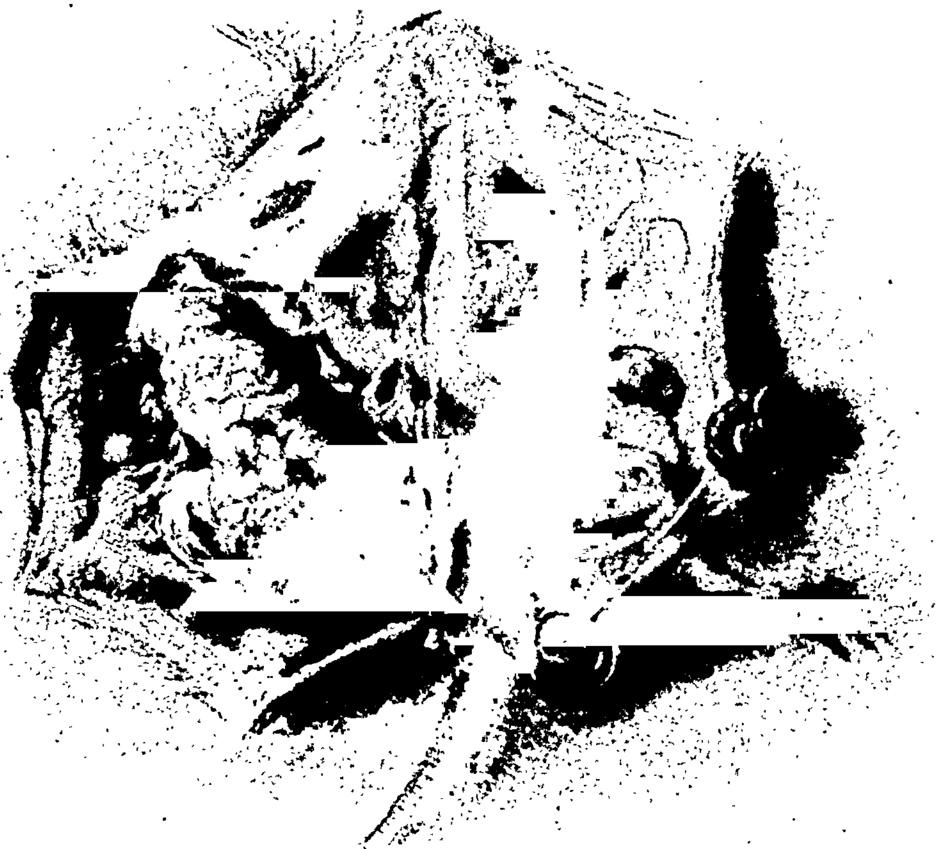
DESCRIPTION OF PLATES

Plate 24

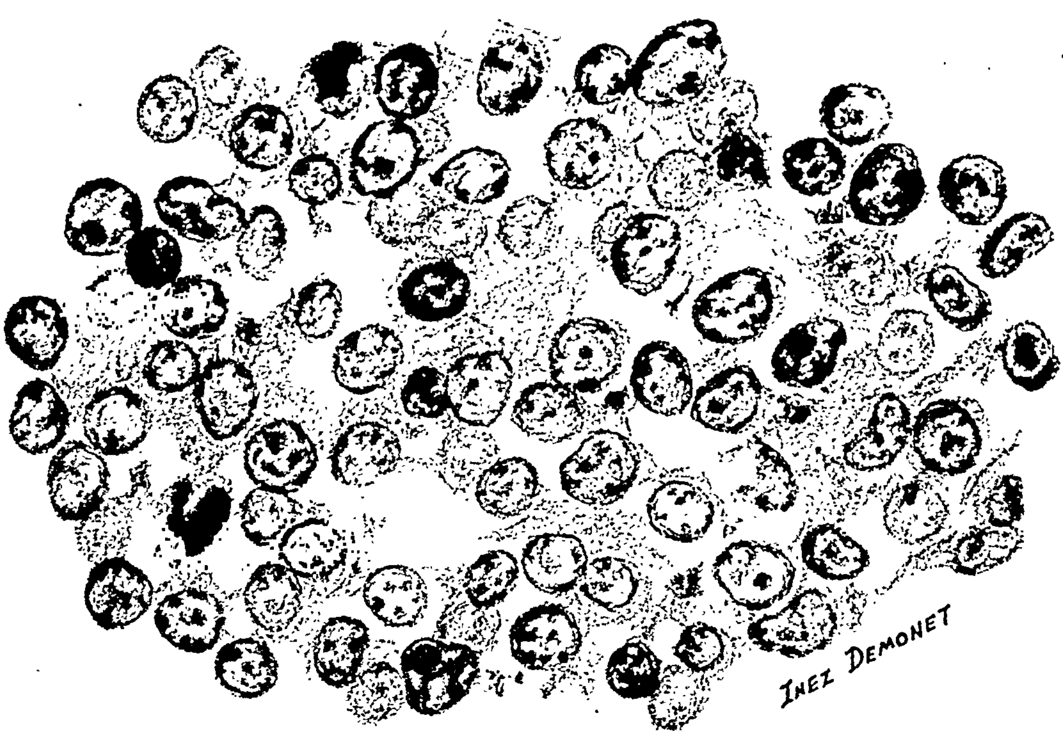
Fig. 1. Late generation transplant of the malignant thymoma. Areas of necrosis appear on the surface, and the opposite inguinal lymph node shows evidence of metastasis. About normal size.

Fig. 2. Drawing of a field from the transplant. In this particular field the cells are loosely arranged; in other areas they were tightly packed. The cells are rounded, 12 to 15μ across, have scant cytoplasm, and their nuclei have a heavy chromatin network. The stroma is scant. Hematoxylin and eosin stain. About $\times$ 900.

1



2



Nettleship

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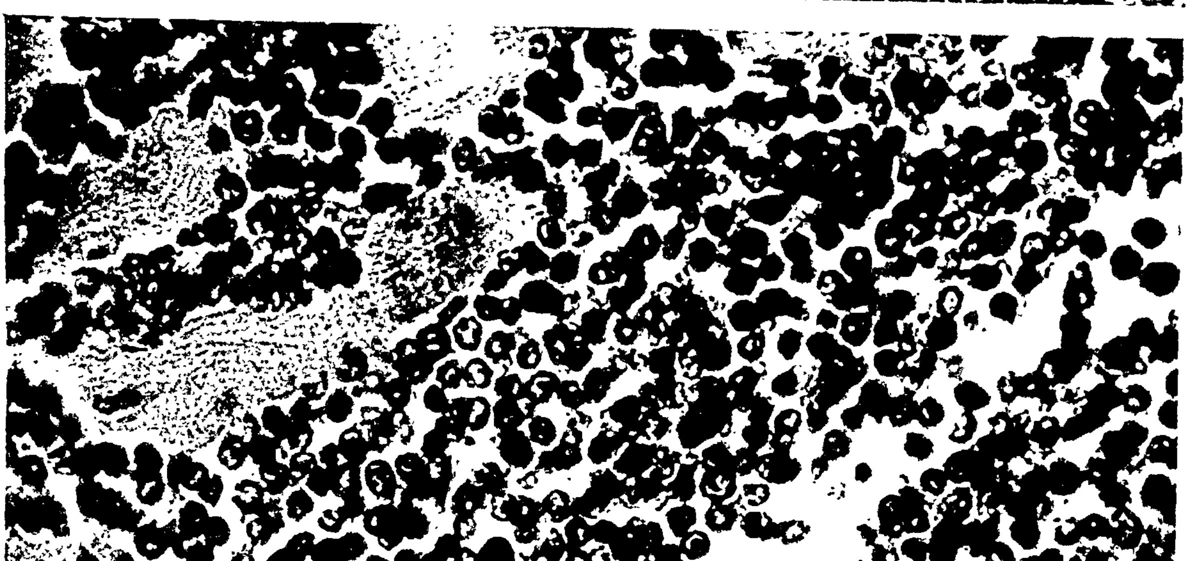
PLATE 25

FIG. 3. Transplant showing the locally invasive character of lymphoma A. The malignant cells have grown into and surrounded muscle fibers. Hematoxylin and eosin stain. × 87.

FIG. 4. Detailed character of the malignant cells. Fibers of striated muscle are at the top and bottom of this field. Hematoxylin and eosin stain. × 1000.



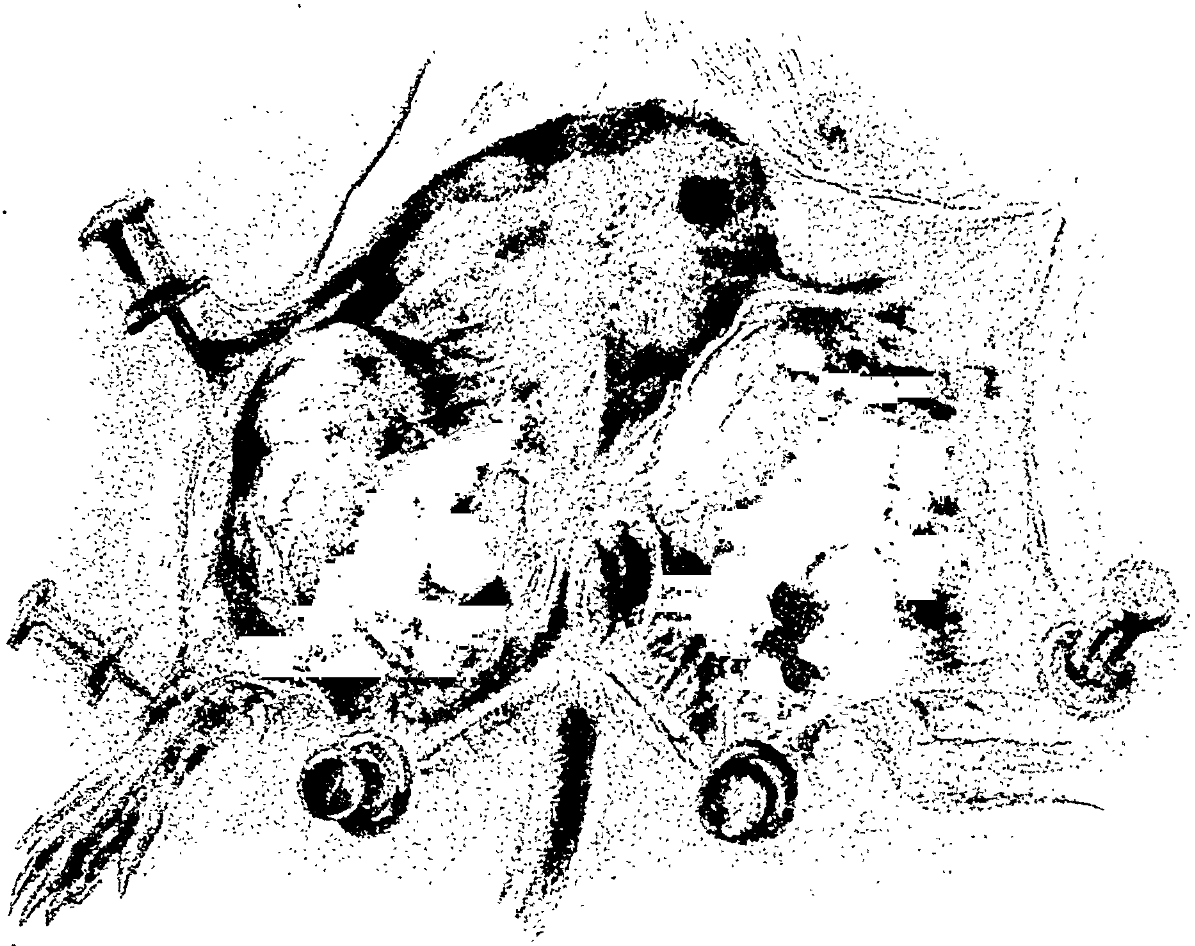
Nettleship

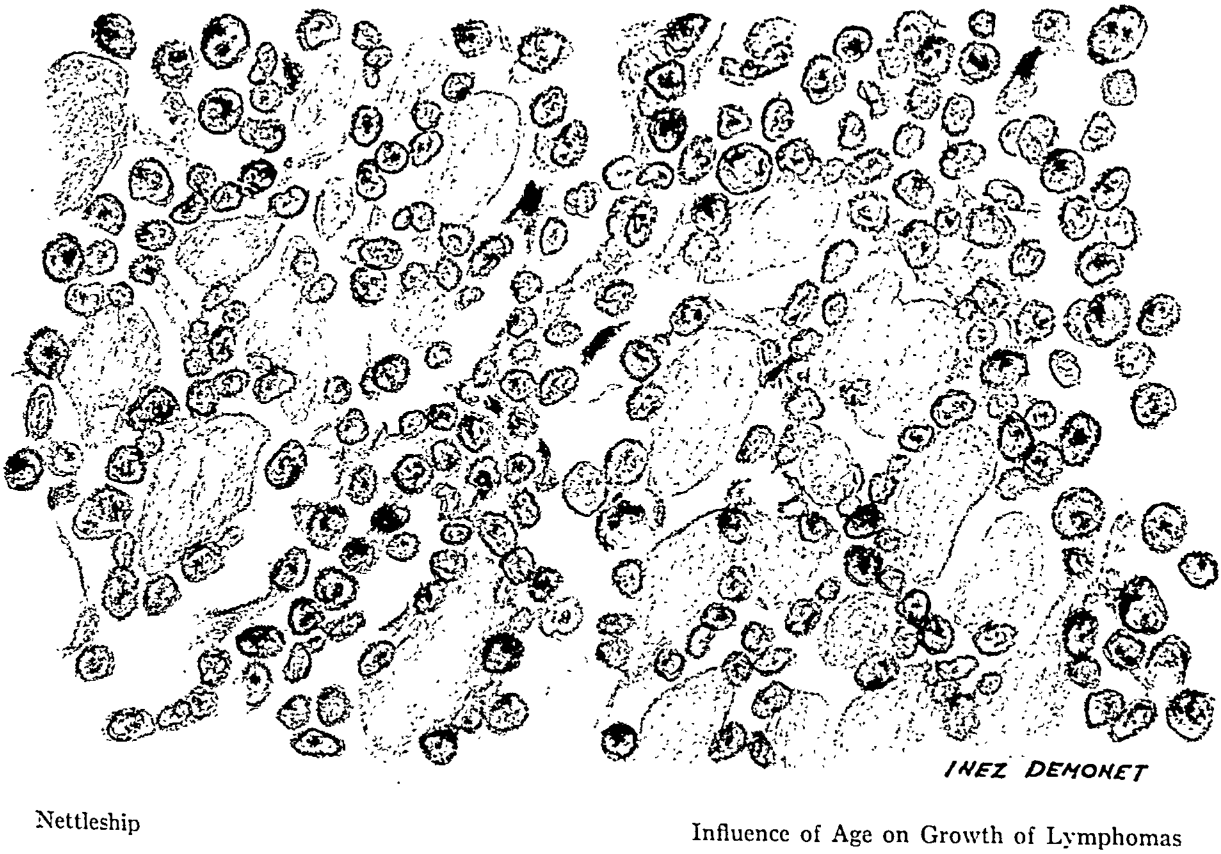
Influence of Age on Growth of Lymphomas

PLATE 26

FIG. 5. Bilateral inguinal transplants. The tumors were at about their limit of growth; they were 2 weeks old and measured between 30 and 40 mm. in longest diameter.

FIG. 6. Drawing of high-power field to show character of cells and local invasion of abdominal muscle wall. Hematoxylin and eosin stain. × 1000.





Nettleship

Influence of Age on Growth of Lymphomas

GROWTH OF A MOUSE LYMPHOMA COMPARED TO NORMAL TISSUE GROWTH *

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One aspect of the growth of a neoplasm is its ability to increase in mass more readily than normal tissues. It is uncertain how rapidly neoplasms accumulate protoplasm in comparison to normal tissues. Such data should help us determine the extent to which normal growth restraints have been lost. Although few accurate observations are available on the rate of protoplasmic accumulation, tumor growth has been considered to take place at about the same rate as that of embryonic tissues.[1]

Additional data on this aspect of tumor growth were obtained by utilizing inbred strains of mice which have transplantable lymphomas. Experimentally, it was but necessary to compare daily weights of growing animals with those of the same age which bore tumors.

MATERIALS AND METHODS

The lymphoma used in this experiment arose spontaneously in C57 × C3H mice. It grew locally when implanted subcutaneously, and only late in its course gave rise to extensive spread and leukemia. Its history and morphology have been described, as well as the technic of its transplantation.[2]

Animals of two age groups were inoculated with this lymphoma. The younger group averaged 12 days and had an average weight of 4.8 gm. The older group averaged 42 days and had an average weight of 17.1 gm., almost four times that of the smaller animals. How these groups of animals grew, with and without tumors, is shown in Table I. The weights at death are given in Table II.

DISCUSSION

Perhaps the most significant fact to emerge from Table I is that in young animals which are growing rapidly the total tissue which they are capable of growing is the same regardless of its normal or neoplastic nature. This in turn points up two facts. The tumor, while it is disorganized in growth, does not in reality grow any faster than the varied tissues of young control animals, but the organs of the animal which is bearing the tumor probably suffer from lack of nourishment (Table II). When the older age groups are examined, we see that the

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animals which bore tumors actually outstripped the controls in weights (31 to 22.4 gm.). In this group, however, the organs of the animals which bore tumors did not suffer to the extent that they did in the younger animals.

TABLE I

Average Weights of Mice in Two Age Groups with and without Tumors

	Average weights, young mice		Average weights, young adults	
Day of experiment	Animals with tumors	Control animals	Animals with tumors	Control animals
	(*gm.*)	(*gm.*)	(*gm.*)	(*gm.*)
1	4.8	5.1	17.0	17.2
3	4.3	4.5	18.1	18.3
4	4.5	4.7	18.3	18.3
6	4.8	5.0	18.1	18.0
8	5.7	5.7	19.0	18.6
9	6.2	6.0	19.1	19.1
11	7.6	7.5	19.4	19.3
14	9.6	9.7	20.0	19.7
17	12.4	12.2	21.3	20.1
20	15.4	14.7	24.0	20.8
22	17.0	15.5	26.2	20.3
26	20.2	17.7	29.4	21.1
29	21.0	19.7	33.2	24.3
40	22.4	22.2	31.0	22.5

Schrek,[1] in 1936, in comparing the Walker rat tumor with embryonic rat growth, concluded that both growth rates could be represented by linear curves, and that their actual rates were of the same order of magnitude. He was impressed, as was I, by the fact that the malignant cell does not appear to be endowed with an excessive capacity for growth.

TABLE II

Weights in Two Different Age Groups Which Bore Lymphomas, as Compared to Litter-mate Controls

Age group	Average initial weights		Average final weights		Average weight gains		Amount of normal tissue gain		Amount of tumor gain
	tumor	control	tumor	control	tumor	control	tumor	control	
(*days*)	(*gm.*)	(*gm.*)	(*gm.*)	(*gm.*)	(*gm.*)	(*gm.*)	(*gm.*)	(*gm.*)	
12	4.8	5.1	22.4	22.2	17.6	17.1	9.6	17.1	8.0
42	17.1	17.2	31.0	22.5	14.0	5.3	1.7	5.3	12.3

SUMMARY

Young mice which grew transplantable lymphomas accumulated approximately the same amount of tissue as their litter-mate controls, and the rate of accumulation was similar. One-half of the total gain in weight in the tumor-bearing animals was in the tumor. Older animals which bore tumors grew more tissue than their litter-mate controls.

Their tumor weights could account for almost all of their weight increase.

Even in young animals, the total neoplastic mass accumulated was never greater in any time period than that grown by heterogeneous tissues in litter-mate controls. The actual growth of the protoplasm in this lymphoma appears to be approximately one-half that of heterogeneous normal tissues.

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PROLIFERATIVE LESIONS IN MULTIPLE MYELOMA WITH SPECIAL REFERENCE TO THOSE OF THE SPLEEN

The Origin of the Plasma Cell*

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Plasma cell myeloma has been considered a local bone tumor (Geschickter and Copeland), a local tumor of reticulo-endothelial origin with or without multiple metastases (Hellwig), or a diffuse proliferation of the reticulo-endothelial system (Ewing). Ulrich has proposed a classification of plasma cell tumors into somewhat similar groups. Further, the type cell has been generally accepted, since the series reported by Christian, as the plasma cell. A widely held view is that these cells arise from lymphocytes (Maximow), but this as well as the relationships of this cell has been the subject of much conjecture (Michels). For these reasons, an examination of portions of the reticulo-endothelial system in cases of multiple myeloma might give information both as to the nature of the disease process and as to the origin and relations of the plasma cell.

Material

The material consists of 12 autopsied cases of multiple myeloma, the total number in the pathology laboratories of the University of California and Mt. Zion Hospitals. Most of the tissues had been fixed in formalin, a few in Zenker's fluid. The material had been stained with hematoxylin and eosin, Giemsa's stain, and in a few cases with malachite green-acridine red or with Wright's blood stain. A summary of the pertinent clinical and post-mortem findings is given in Table I.

Findings

The clinical and hematologic aspects will not be further amplified. These have been covered by numerous authors (Ulrich, Wintrobe and Buell, Osgood and Hunter, Churg and Gordon). However, anatomic findings in selected organs will be described in detail.

The spleen was enlarged in all but one of the cases in which the organ was weighed, and the weight of this spleen was near or beyond the upper limit of normal for the patient's age (case C. D., 150 gm.). Microscopically, malpighian follicles were preserved in all of the cases and even in the spleen most enlarged (case F. B.) the follicles were still found as small lymphoid islands separated by wide areas of plasma

* Received for publication, April 3, 1944.

cells. The lymph follicles were of quite usual appearance as is shown in the illustrations from these cases. Likewise, all of the spleens showed plasma cells. The site of plasma cell proliferation could be determined only in those spleens which were well preserved, *i.e.*, in 7 cases; in the 5 showing marked post-mortem alteration no conclusions could be reached as to the relationships of these cells to other finer structures. Post-mortem alteration appears to be the limiting factor in the cases in which this specific lesion could not be made out. The lesion is shown in the accompanying plates and consists of a local proliferation of plasma cells which are either replacing a sinus endothelial cell, or in other cases are superimposed into the sinus lumen upon an intact lining cell. The appearance suggests that endothelial cells divide in two planes, in one plane each producing a littoral cell and beside it a plasma cell. The latter replaces the flat endothelial cell which would normally be in this location. In the other plane the continuity of the sinus lining is maintained by the littoral cells and the plasma cells are superimposed into the vascular lumen on the cells of origin. Most of these plasma cells are still attached and occasionally form clusters hanging into the blood stream. Elsewhere they are free within a small channel, apparently broken from their site of attachment. The lesion will be discussed later.

The lymph node involvement was somewhat similar in that all of the lymph nodes examined contained at least some plasma cells. Some nodes, however, were massively replaced, showing loss of architecture and invasion of the capsule by sheets of plasma cells. Others, even in the same cases, were less completely replaced and these are considered in particular, for, in all, the masses of plasma cells formed bands between the lymph follicles. The lymph follicles with their germinal centers remained as the last normal landmarks and were separated by wide zones of tumor cells. Thus, plasma cells were present in the interfollicular portions of the nodes and the areas of lymphoid proliferation were unaffected till later, or showed but a few stray cells of the plasma cell variety. No relation of plasma cells to sinus lining could be established, the plasma cells usually forming sheets obliterating littoral cells and all sinus structures.

The liver was definitely enlarged in 6 cases. Three of these livers showed infiltration of leukemic type with plasma cells. The liver in another case (B.K.), though not greatly enlarged, showed early infiltration of the same variety. There were plasma cells in large collections in the portal areas and in groups throughout the remainder of the lobule. The remaining livers were notable only for their lack of involvement and, in all, the Kupffer cells were clearly distinguished and of usual appear-

TABLE I

Summary of Findings in 12 Cases of Multiple Myeloma

Case	Age	Sex	Duration according to history	Serum protein (gm./100 cc.)			Bence-Jones proteinuria	Red blood cells	Hemoglobin	White blood cells	X-ray*	Liver		Bone marrow infiltration	Lymph node infiltration	Spleen	
				Total	Albumin	Globulin						Weight	Leukemic infiltration			Weight	Lesion noted
			(mos.)					(000,000)	(gm.)	(000)		(gm.)				(gm.)	
N. S.	64	F	10	17.6	1.6	16.0	o	1.92	6.6	13.0	o	2100	o	+	+	590	o
L. P.	54	M	11	8.6	4.9	3.7	+	3.09	9.9	6.2	+	2065	o	+	+	390	o
F. B.	59	M	3	6.6	4.2	2.4	+	2.85	9.5	10.0†	+	2720	+	+	+	760	+
E. G.	53	M	4				+	3.07	8.7	6.5	o	"Normal"	o	+	+	450	+
I. W.	55	M	5				o	2.00	5.4	10.3	o	"Large"	o	+	+	"Normal"	o
M. B.	70	F	12	6.0	3.4	2.6	o	3.71	9.8	14.9	o	"Normal"	o	+	+	"Normal"	+
B. K.	55	M	14	13.3	2.9	10.4		1.35	4.8	10.0	o	1820	+	+	+	280	+
R. B.	53	M	7	14.2	2.5	11.7	+	3.80	6.0	11.2	o	"Normal"	o	+	+	"Normal"	o
C. D.	50	M	48	7.8	5.6	2.2	+	2.40	7.8	8.3	+	1525	o	+	+	150	o
R. J.	52	F	120‡			§		1.23	4.8	3.4	+	2435	+	+	+	230	+
H. H.	65	M	18	6.2	3.1	3.1	+	1.80	5.2	6.3	+	"Large"	+	+	+	"Large"	+
H. M.	44	M	22	7.4	4.7	2.7	+	1.75	6.0	6.6	+	1720	o	+	+	300	+

* Or radioactive phosphorus.
† Five per cent plasma cells in differential leukocyte count.
‡ Thigh amputated for local tumor 4 years before death.
§ Terminal hyperglobulinemia suggested by marked auto-agglutination of blood.

ance. This infiltration is of interest in that it is not the type usually characteristic of lymphatic leukemia, but rather resembles the distribution often seen in the monocytic or the myelogenous types.

Bone marrow from multiple regions was examined and all specimens showed numerous plasma cells. In general, these cells replaced all marrow elements, including the vascular channels. Usually the bone trabeculae were small. This massive involvement illustrates the diffuseness of the process, but obliterates any relationship of the plasma cell to a specific cell as precursor.

Discussion

A. The Origin of the Plasma Cell

In this disease two organs give clues as to the origin of the plasma cell. First, the spleen suggests by its appearance that it is a site of local proliferation of plasma cells. Maximow described the splenic sinuses as cylindrical meshes lined by squamous reticular cells, a part of the general reticular or histiocytic framework. Knisely believed that this system is closed. In plasma cell myeloma the cells that are proliferating into the sinus spaces appear to be derivatives of these lining cells. Their mode of proliferation is like that described by Doan, Cunningham and Sabin for megaloblasts and for clasmatocytes, that is, they hang as single cells or as clusters into the vascular stream. The cells actually form a part of the vessel wall and do not appear to have been lodged there by chance from the blood stream, or to have reached this site by invasion from an extravascular source. However, since the littoral cells are apparently closely related to the cells of the diffuse framework of the pulp, it is quite possible that plasma cells are arising also in the perisinusoidal tissue. This origin from the framework reticular cells might account for the plasma cells distributed throughout the spleen. Likewise, the preservation of intact lymph follicles, even with extensive plasma cell involvement of the spleen, speaks against the lymphocytic origin of these cells.

Second, the lymph nodes suggest likewise a histiocytic origin of the plasma cell. Downey described the lymph sinuses as lined by squamous histiocytic cells only near the afferent and efferent radicals. The finer sinuses are directly continuous with the general reticulum of the pulp. The lymph nodes in this group of cases showed diffuse proliferation in sinus areas with lymphatic tissue remaining intact. The diffuse nature of this growth in lymph nodes, where plasma cells are not seen arising from sinus endothelial cells as they are in the spleen, might well be due to the absence in lymph nodes of a completely closed system of littoral cells. Recently, Parsons has shown the new growth of plasma cells

from littoral cells in lymph nodes of mice treated with x-ray and with carcinogens and has suggested the histiocytic origin of plasma cells. This is in accord with the lymph node involvement in these cases. It seems unlikely that the plasma cells in lymph nodes are of metastatic origin from a local tumor, because of the involvement of lymph nodes in many areas and their progressive replacement, even in the absence of metastatic nodules in such common sites of spread as lung and liver.

The liver is of interest for several reasons. First, the absence of changes in the Kupffer cells fits well with the contention of Sabin that these cells are completely differentiated derivatives of the reticulo-endothelial system. It is also of interest that when infiltration of the liver does occur (cases F. B., B. K., R. J., and H. H.), the cell distribution is similar to that commonly seen in myelogenous and monocytic leukemia, rather than to that of lymphatic type. The distribution in the cases reported by Osgood and Hunter and by Churg and Gordon was also of this type. In this connection, another suggestive resemblance of this tumor to tumors of histiocytic, rather than lymphoid, relations is brought out in the series of malignant lymphomas studied by Gall and Mallory. In that series clasmatocytic lymphomas were the only tumors of the group which involved bone with any degree of regularity.

In review of these anatomical relations, the lymphoid origin of the plasma cell is contradicted by the persistence of lymphoid follicles in the spleen and lymph nodes even when these lymphoid masses are separated by wide areas of plasma cells. Likewise, the type of hepatic infiltration, when it occurs, speaks against a lymphocytic relationship. On the other hand, an origin from histiocytic type cells is indicated by the sites of proliferation in spleen and in lymph nodes, by the type of hepatic infiltration, and is suggested by the invasion of bone.

B. The Nature of the Process

There are numerous reports of local plasma cell tumors, and extramedullary examples have been gathered recently by Hellwig. Yet all of the cases in the series reported here showed extensive involvement of the reticulo-endothelial system, the histiocytic or reticular portions in particular. This was irrespective of their original onset, for one that showed widespread involvement (case R. J.) was treated for a local bone tumor by amputation of the thigh 4 years before death. It is true that these cases are, in a way, a selected series in that all were fatal. However, it would seem that this disease must be considered at least potentially diffuse, no matter how localized the original lesion may seem.

C. *Relation of the Plasma Cell to Hyperglobulinemia*

It would appear, then, that the plasma cell in origin is related to the monocyte, the clasmatocyte, or macrophage of Cunningham, Sabin and Doan, or to the tissue histiocyte of Maximow. These are the cells which Sabin has shown to be concerned directly in the formation of antibodies. The occurrence of the plasma cell in physiologic states of increased antibody-globulin formation and in conditions of abnormal excess globulin production suggests that this is a valid functional relationship as well. Bing and Plum, and, more recently, Kagan have discussed hyperglobulinemia and have shown that the most common causes of this condition are chronic infections, particularly tuberculosis, syphilis, leprosy, lymphogranuloma venereum, subacute bacterial endocarditis and kala-azar. These, according to Bing, are associated with changes in the reticulo-endothelial system, notably with the accumulation of plasma cells. An interesting report in this connection is that by Bjorneboe and Gormsen, who produced tissue infiltration of plasma cells, especially in the spleen but also in the liver and in other organs, by repeated immunization of rabbits with killed pneumococci. These animals showed definite elevation of serum globulin, which was proportional both to the degree of plasma cell infiltration and to the agglutination titer of the serum.

Among the noninfectious causes of hyperglobulinemia, multiple myeloma is listed first. Only 2 of the 9 cases of this series in which the serum globulin was determined showed normal serum globulin levels (considering 2.58 gm. per 100 cc. as the average normal as given by Peters and Van Slyke). In such cases Bing and Plum believe the type cell to be more mature and less rapidly proliferating. These authors stated that the highest values are found in those cases with extremely immature cells. Sabin has shown that globulin release by clasmatocytes is associated with the shedding of cell cytoplasm into the blood stream, a process for which maturity is perhaps unnecessary. However, this series of cases is too small to correlate the duration of the illness with serum globulin level and this point must remain for further investigation. Hyperglobulinemia occurs also in leukemias, notably monocytic, but there are rarer reports of increases in myeloid and lymphatic types as well (Bing and Plum, Kagan).

No discussion is attempted here of the renal lesion or of Bence-Jones proteinuria. These aspects are discussed in particular by Bell, and by Forbus, Perlzweig, Parfentjev and Burwell.

Summary

A lesion of the reticulo-endothelial system in multiple myeloma is described, in particular as it is found in the spleen and lymph nodes.

In the spleen this lesion consists of the intrasinusoidal proliferation of plasma cells from sinus lining. In addition, plasma cells are found throughout the red pulp. In lymph nodes plasma cells proliferate in the interfollicular tissue. Lymphoid structures remain intact in both these organs. The presence of a closed system of littoral cells in the spleen, in contrast to that of lymph nodes, is suggested as the explanation for the localization of plasma cells in relation to sinus lining only in the former organ. These lesions, as well as the distribution of leukemic infiltration when the liver is involved, and the tendency to involve bone, suggest that plasma cells do not arise from lymphocytes or their immediate precursors, but that they arise, at least in this disease, from tissue histiocytes. It thus appears that plasma cell myeloma is more closely related to diseases of monocytic or clasmatocytic type than of lymphoid type. It is pointed out that the disease, multiple myeloma, at necropsy consists of a diffuse proliferative process involving the entire reticulo-endothelial system, regardless of its predominant skeletal or local onset. In origin the plasma cell is related to those cells which have been specifically shown to be concerned in antibody formation. Previous observations on the occurrence of the plasma cell suggest that it, too, forms globulin and is concerned with the formation of antibody globulins and in abnormal states with hyperglobulinemia.

It is a pleasure to acknowledge the numerous suggestions offered by Dr. James F. Rinehart throughout the course of this study and the review of the manuscript by Dr. Gerson R. Biskind.

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DESCRIPTION OF PLATES

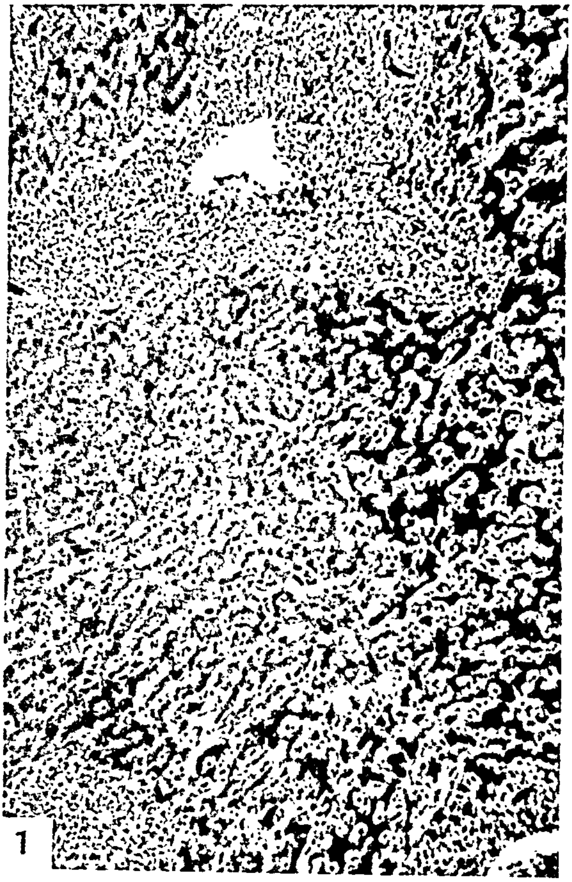
PLATE 27

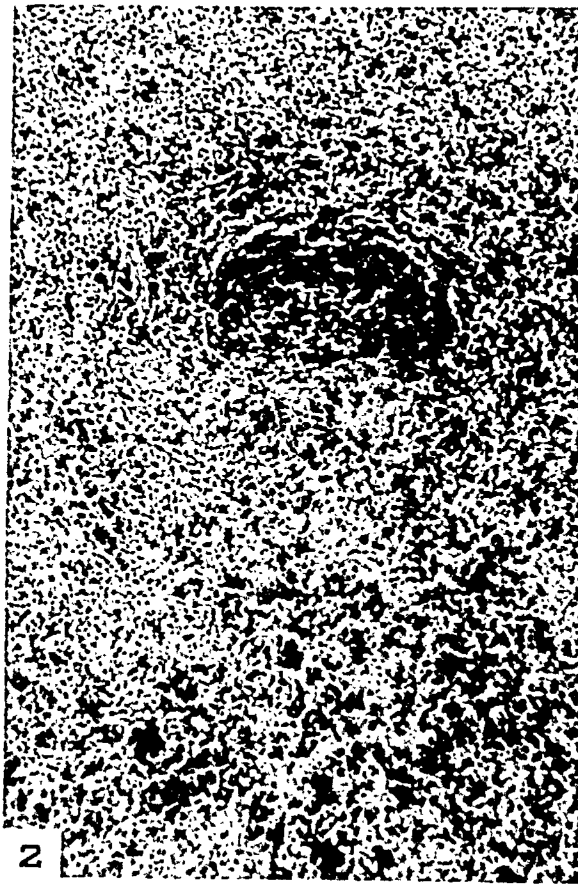
FIG. 1. Infiltration of the liver (case F.B.) showing plasma cells throughout the lobule as well as in portal areas. The same type of infiltration was present in the other three livers involved (cases B.K., R.J., H.H.). × 75.

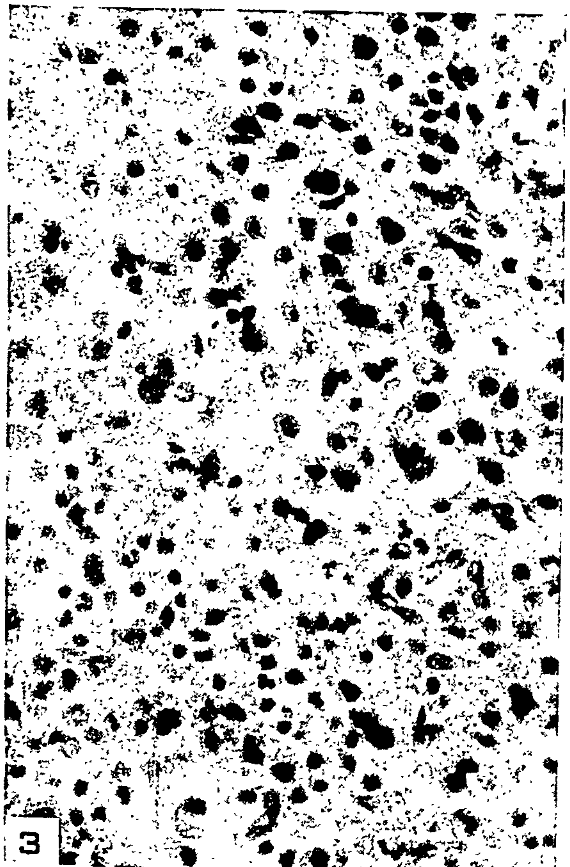
FIG. 2. Spleen (case R.J.) showing lymphoid tissue surrounded by plasma cells. × 95.

FIG. 3. The same spleen as in Figure 2 to show several sinuses lined by plasma cells. The one in the upper center is lined in part by flat littoral cells and in part by plasma cells. × 580.

FIG. 4. The same lesion as in Figure 3 in greater detail. × 1160.







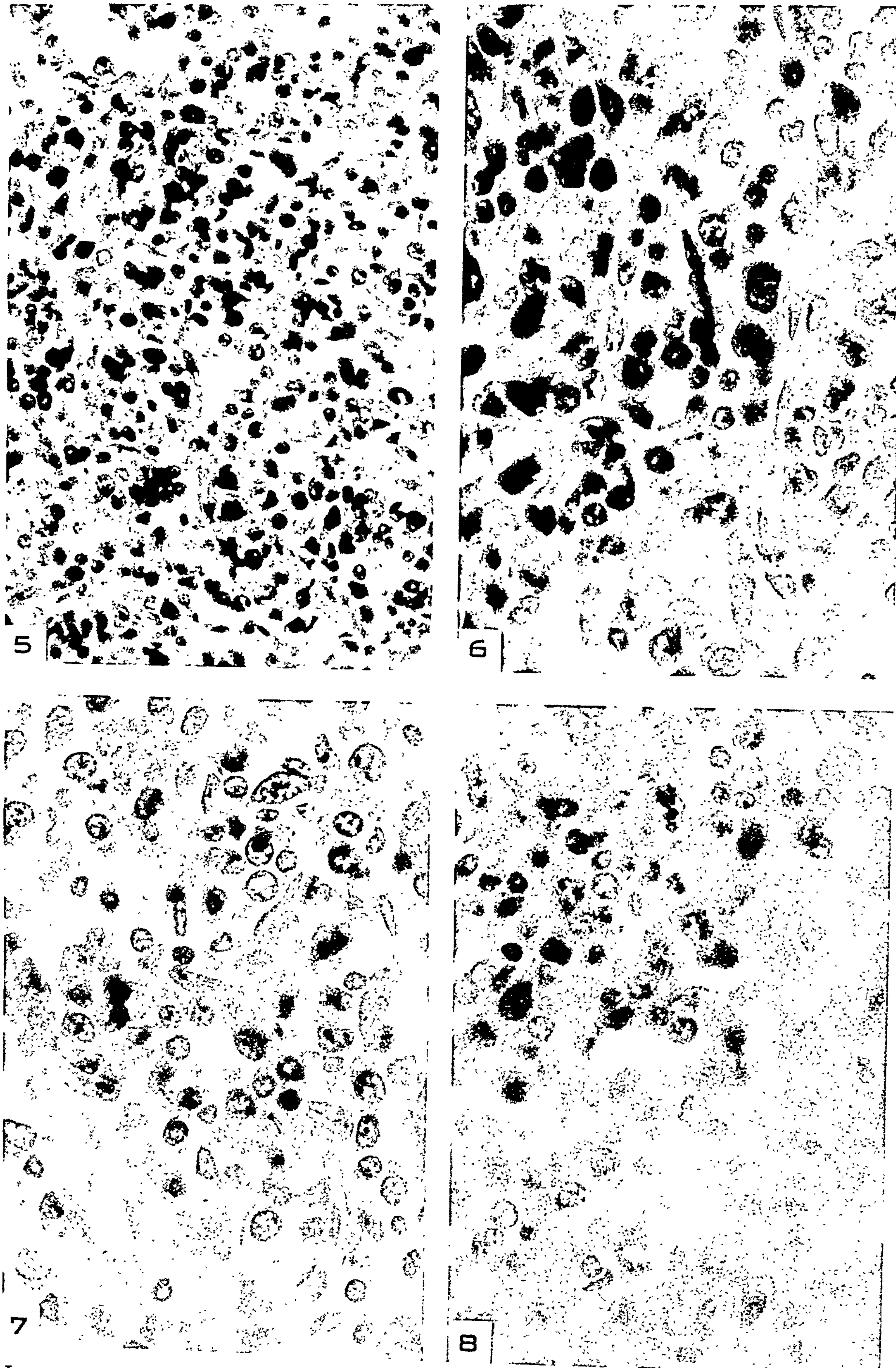
Lowenhaupt

Proliferative Lesions in Multiple Myeloma

PLATE 28

FIG. 5. Spleen (case F.B.) showing several sinuses with a proliferated lining of the same type as shown in the preceding figures. There are numerous plasma cells in the perisinusoidal tissues. × 580.

FIGS. 6, 7 and 8. Details of sinuses of the same spleen (case F.B.). Plasma cells project into the blood stream. Some replace littoral cells and some are superimposed on them. × 1160.



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Proliferative Lesions in Multiple Myeloma

PLATE 29

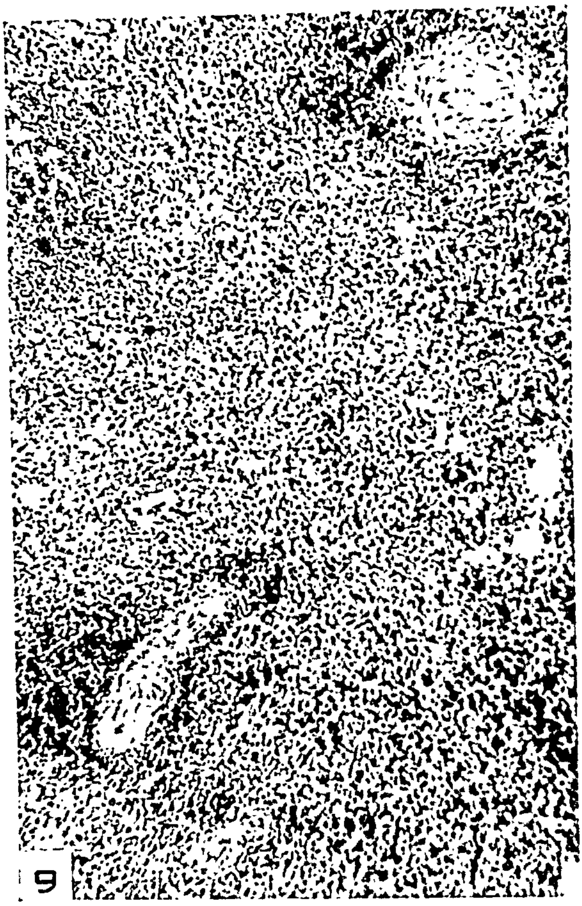
FIG. 9. Spleen (case H.H.) with malpighian follicles separated by masses of plasma cells. × 95.

FIG. 10. The same spleen as in Figure 9 with many sinuses lined by proliferated plasma cells. Some form clusters projecting into the lumen. × 580.

FIG. 11. A single sinus from the same spleen as Figures 9 and 10 to show this lesion. × 1160.

FIG. 12. Spleen (case B.K.) with persistent malpighian corpuscles. × 95.

FIG. 13. Details of the same spleen (case B.K.) with cells attached at their sites of proliferation. × 1160.



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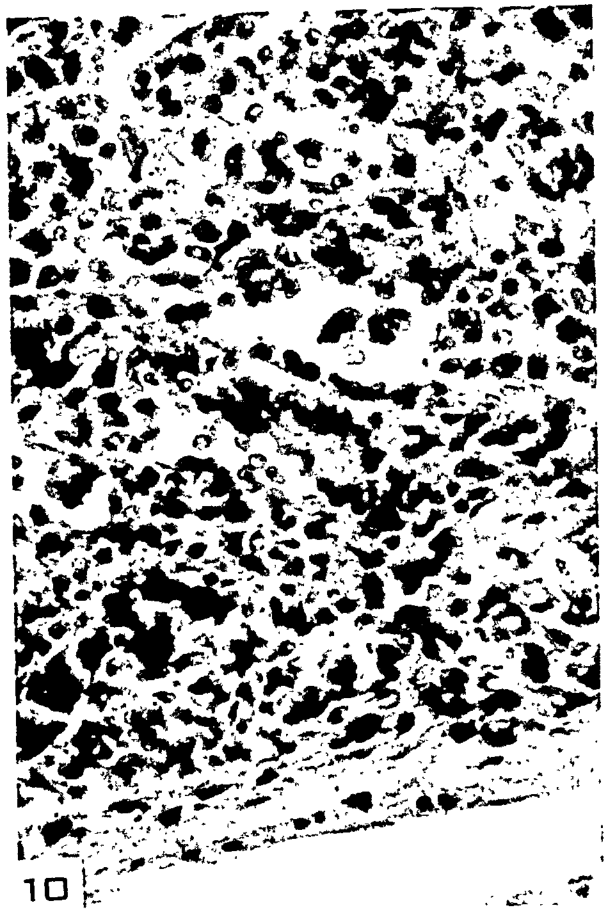

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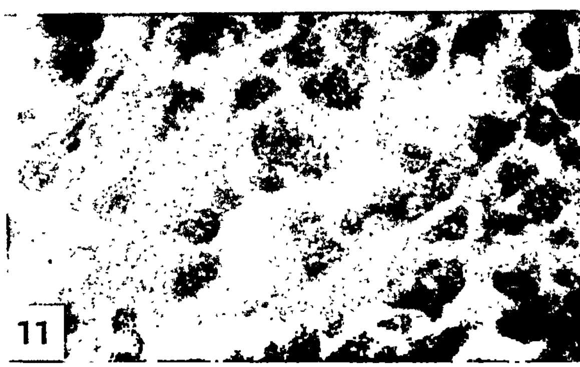

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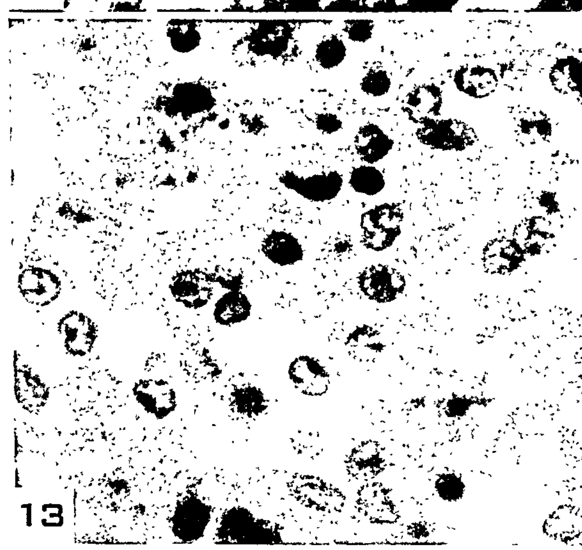

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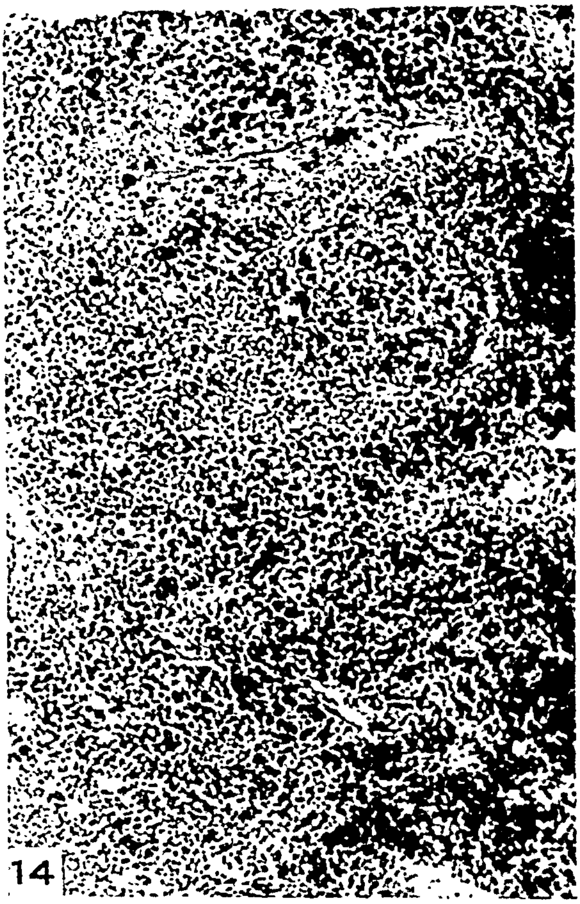
Proliferative Lesions in Multiple Myeloma

PLATE 30

FIGS 14 and 15. Lymph nodes (cases M.B. and B.K.) with persistent lymphoid follicles separated by masses of plasma cells. $\times$ 95.

FIGS. 16 and 17. Lymph nodes (cases R.J. and E.G.) with complete destruction of architecture and invasion of capsule by plasma cells. Other nodes from these same cases were of the type shown in Figures 14 and 15. $\times$ 95.



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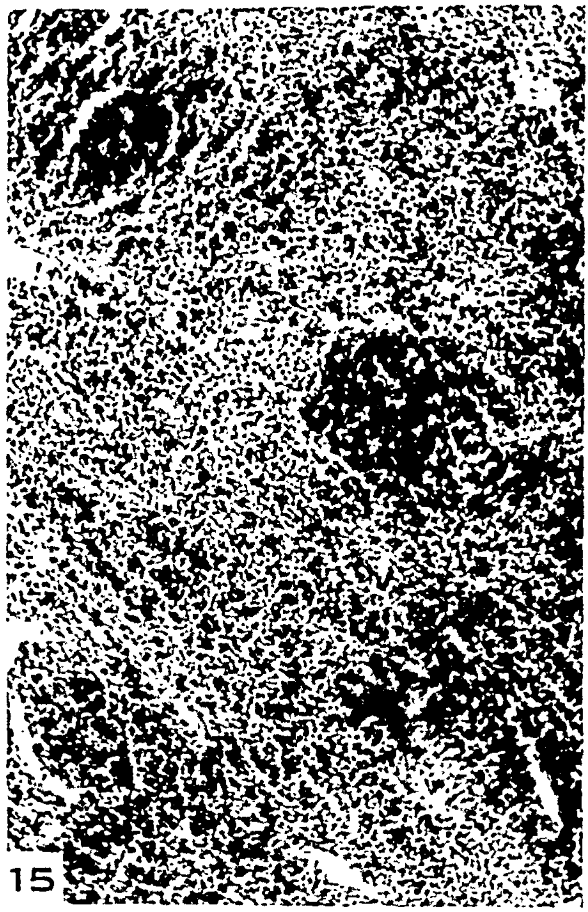

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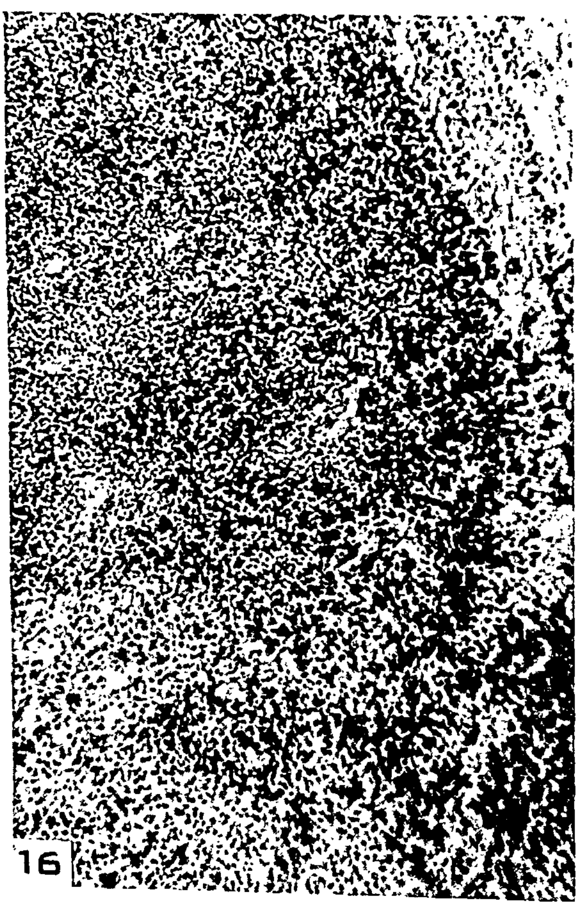

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Proliferative Lesions in Multiple Myeloma

CONGENITAL CYST OF THE MYOCARDIUM *

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An epithelium-lined cyst of the heart is a rare lesion. Such a lesion has interesting theoretical implications as to origin, and so the presentation of an additional case seems warranted.

Report of Case

The patient, G.F. (A-42-252), was a white male, 44 years old, who had worked as a counterman. He was admitted on July 16, 1942, with severe precordial pain radiating to the left arm and neck for a period of 3 hours. Mild precordial discomfort had been present for the past few days.

On admission, the patient was vomiting. He showed a gray hue to his skin and was sweating. Physical examination revealed nothing of note. The heart sounds were fair, with a regular sinus rhythm of 60. The blood pressure was 110/80 mm. of Hg. The respirations averaged 24 per minute. The urine was negative for albumin and glucose.

In spite of morphine, nembutal, caffeine and sodium benzoate, the patient expired on July 17, 1942, with the final clinical impression of an acute coronary occlusion.

The autopsy confirmed the clinical diagnosis. A fresh thrombus was found in the descending branch of the left coronary artery, approximately 1 cm. below the ostium. Both coronary arteries showed marked atherosclerosis throughout. Microscopically, areas of necrosis and also of old fibrosis were found in the myocardium. The heart weighed 280 gm.

A small cyst situated in the center of the left ventricular wall was an incidental finding (Fig. 1). The cyst measured 0.9 cm. in diameter. Its cavity was rounded and sharply delimited. The lining showed a thin, glossy smooth layer of translucént tissue. The contents of the cyst were gelatinous and translucent, with a pale greenish tint. The cyst was located well within the left ventricular musculature, failing to reach the epicardium or the endocardium. Its lower border was located about 3.5 cm. from the apex. The cyst was completely surrounded by cardiac muscle, and did not occasion any discernible bulging of the muscle toward either the epicardial or the endocardial surface. No external manifestation of its existence was present, and the structure was found only because of a fortunate routine section through its center.

On microscopic examination, an inner layer of epithelial cells was seen. The epithelium was tall columnar for the most part, with a shorter, more cuboidal appearance in some areas. The epithelial lining was distinctly ciliated (Fig. 2). The cilia were numerous, closely placed and of uniform height for each cell. In the shorter cells, the cilia

* Received for publication, April 13, 1944.

were not so tall, but were proportionately more prominent, and, for some cells, occupied as much as one-half the full height of the cell. Most of the cilia of the taller epithelium measured less than one-third or one-fourth the height of the cell structure. The cilia were inserted on a sharp cell border, which appeared as a narrow, linear, condensed eosinophilic zone. The cytoplasm was distinctly granular and eosinophilic. The nuclei were situated in the basilar portions of the cells and were elongated and oval. The nuclei showed some variation in size, but the range of variation in both size and shape was rather narrow.

Discussion and Comparison with Other Cases

Very few similar instances of epithelial cyst formation within the myocardium could be found in the literature. Five of the published cases (Stoeckenius,[1] Kolatschow,[2] Davidsohn,[3] Yamauti [4] and Bayer [5]) represent lesions that are very similar to the one presented here. A sixth case by de Chàtel,[6] and two other instances reported by Bayer, differ in many respects. The last three will be discussed separately. There were several features common to the first five cases. They were all situated in the left ventricle of the heart, near or in a papillary muscle. Yamauti and Davidsohn described unilocular formations like the one observed by us, while the cyst reported by Stoeckenius showed subdivisions into several smaller cavities "due to extension between bundles of muscle fibers." Kolatschow described two smaller confluent structures in addition to the oval main cyst. He did not state clearly whether he dealt with one large cystic structure with an irregular invaginating or undulating wall, or with three separate, closely placed cysts. The cysts described by Stoeckenius, Yamauti and Bayer, as well as the lesion presented here, were situated completely within the myocardium. The reports of Kolatschow and Davidsohn note that the cystic structure projected partially into the ventricular cavity.

The ciliated epithelial lining represents an important finding in the five cases with identical features. Its significance lies in the clue it gives to the possible origin of these cystic structures. In the cases of Kolatschow [2] and Davidsohn,[3] as well as in this instance, the contents of the cyst were gelatinous. Yamauti [4] described the contents as homogenous and "pale purple, with hematoxylin-eosin stain." Stoeckenius [1] noted a finely granular meshwork with some clumped desquamated epithelial cells in the contents. Bayer [5] described rounded, albuminoid formations in the lumen. The epithelium is described by Davidsohn as existing in two layers—an outer cuboidal and an inner higher and densely ciliated layer. The cytoplasm in his specimen was eosinophilic

and granular. The nuclei were dark, hyperchromatic and oval, some being highly irregular in shape. Yamauti found "one layer of cuboidal, distinctly ciliated epithelium, with almost oval nuclei, situated in the center of the cells." Kolatschow's case showed columnar ciliated epithelium. He failed to mention the number of layers, but his photomicrograph shows only one distinct layer of epithelial cells. He described the nuclei as "enlarged [ausgedehnten]" possibly meaning elongated, and stated that "they are smaller and more rounded toward the base." Bayer found the cyst lining to consist of one layer of high columnar epithelium with oval, deep-staining nuclei in the lower two-thirds of the cells. Stoeckenius reported columnar cuboidal and flat cells, all showing abundant cilia. Davidsohn, and also Bayer, suggested that the difference in the height of the cells in the last mentioned case may represent an artifact produced by the thickness of the section. The presence of cilia on all of the cells favors this interpretation. The cytoplasm is described by Stoeckenius as "granular, sometimes vacuolated." He described the nuclei as vesicular, elongated or rounded, sometimes hyperchromatic and rod-shaped.

Except for Stoeckenius,[1] all authors mention a thin fibrous wall situated outside of the epithelium. Such a fibrous wall was present in our specimen (Fig. 2). Davidsohn[3] found a distinct homogeneous eosinophilic basement membrane. Bayer[5] found none. A hyaline refractile basement membrane was not made out in hematoxylin and eosin stained sections in our case, but was seen after use of phosphotungstic acid hematoxylin and van Gieson's stains, and best with Verhoeff's elastica stain. No smooth muscle was present, as noted by Bayer. Included atrophic heart muscle fibers were made out, deeply imbedded in the collagenous stroma of the fibrous wall of the cyst.

In none of the reported cases were the cysts found to have clinical significance. In none of them were they diagnosed antemortem. Davidsohn[3] had only negative evidence to support an opinion of a causal relationship for the blowing systolic murmur and thrill which were heard at the base of the heart and then at the apex in his case.

Discussion of Origin

Cystic structures in the heart wall have received different explanations for their origin. All authors agree that the lesion represents a congenital anomaly. According to Stoeckenius,[1] the cyst is due to persistence of the embryologic sponge-like structure of the myocardium, corresponding to the stage of development in fish and amphibians. The cystic structures of this stage are all communicative with the main cavity of the heart, and are lined by endothelium. He assumed that

the endothelium at this stage is multipotent and can differentiate to become columnar and ciliated. The flat cells he observed reminded him of endothelial cells of the epicardium. This theory has the objection that it derives the ciliated columnar epithelium from endothelial cells. As noted above, Davidsohn [3] and Bayer [5] explained the flat cells described by Stoeckenius on the basis of a histologic artifact. This viewpoint would seem to be strengthened, as suggested by Davidsohn, by the fact that all of the cells showed cilia.

Kolatschow [2] presented another hypothesis. He placed the origin from the external part of the myocardial plate, which is the accepted origin for the epicardium, by a process of invagination into the internal plate, which is to become the myocardium proper. This proposal also derives a ciliated columnar epithelium from the mesenchyme. Bayer's [5] case of endothelium-lined cyst and diverticulum may be explained thus. The island of hyaline cartilage found in the wall of this structure may be mesenchymal, though metaplastic origin from epithelium is accepted for this tissue. We have often seen the serous epicardial covering differentiate or undergo metaplasia into rather tall, columnar cells. A cuboidal appearance of the epicardial cells is not at all uncommon. We do not recall, however, any cilia on such an altered epicardial surface layer. Yamauti [4] did not go into details concerning the origin of these cysts. Davidsohn [3] compared the structure with esophageal cysts. He noted a complete absence of embryologic data that would offer an adequate explanation for the cardiac cyst on this basis. Bayer [5] referred the origin of the cystic structure with ciliated epithelium in his case to displaced tissue of the bronchial tree. He pointed out that "as yet no relations between the heart and bronchial tree are found embryologically, but may exist." In favor of the concepts of Davidsohn and Bayer is the fact that, whereas the mesoderm ordinarily fails to produce ciliated epithelium, the entoderm, which is the source of origin for the bronchial tree and esophagus, does do so.

There exists in the early embryo an ideal stage for the heterotopic inclusion of entoderm by the mesoderm which forms the heart. This is seen in the chick embryo when the original flattened germ layers are folded ventrally to establish the body form, the gut lumen and the single heart. The bilateral cardiac Anlagen are in the region of the head at this time, and in intimate contact and partly enclosed by the endoderm to form the foregut. It is during the fusion of the bilateral cardiac primordia to form a single chamber at this juncture that the circumstances seem most favorable for such inclusions. Under this concept, the ciliated cyst of the myocardium represents a *sequestration cyst involving entoderm* in contrast to the mediastinal dermoids, which

may be derived from the ectodermal layer. De Chàtel[6] suggested this mechanism of origin for his dermoid cyst.

The entoderm can give origin to squamous epithelium, as in the esophagus, and this heaping up of the epithelial cells occurs early in the embryogenesis of this organ. In some of the lower forms, such as Amphioxus, the esophagus is actually lined by ciliated columnar epithelium. This would mean that the squamous epithelial cysts and the so-called esophageal cysts of the mediastinum can also take origin from the entodermal foregut. It would simplify our problem here if we could assume that the squamous epithelial cysts represent inclusions occurring *later* in the embryonic development of this region. The ciliated cysts represent, then, inclusions occurring very early in the embryo, probably at the time of the formation of the primitive foregut. The case of de Chàtel,[6] with both squamous and columnar epithelium in the wall of the same cyst, favors such theoretic considerations. By this concept, the esophageal cysts and the cardiac cysts are closely related, if not identical in origin. No direct factual evidence for any of these theoretic propositions is known to exist in the human embryo.

Conclusion

A cyst of the myocardium, lined by ciliated columnar epithelium, is described, and five similar cases in the literature are reviewed. Consideration of the theoretical implications as to origin results in the suggestion that such cysts arise through the heterotopic inclusion and sequestration of entoderm during the formation of the primitive foregut and single-chambered heart.

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[*Illustrations follow*]

DESCRIPTION OF PLATE

PLATE 31

FIG. 1. Sectioned left ventricular wall, with transected cyst indicated by arrows.

FIG. 2. Photomicrograph of cyst wall showing the ciliated columnar epithelium which lined the cyst, and adjacent stroma. × 400.

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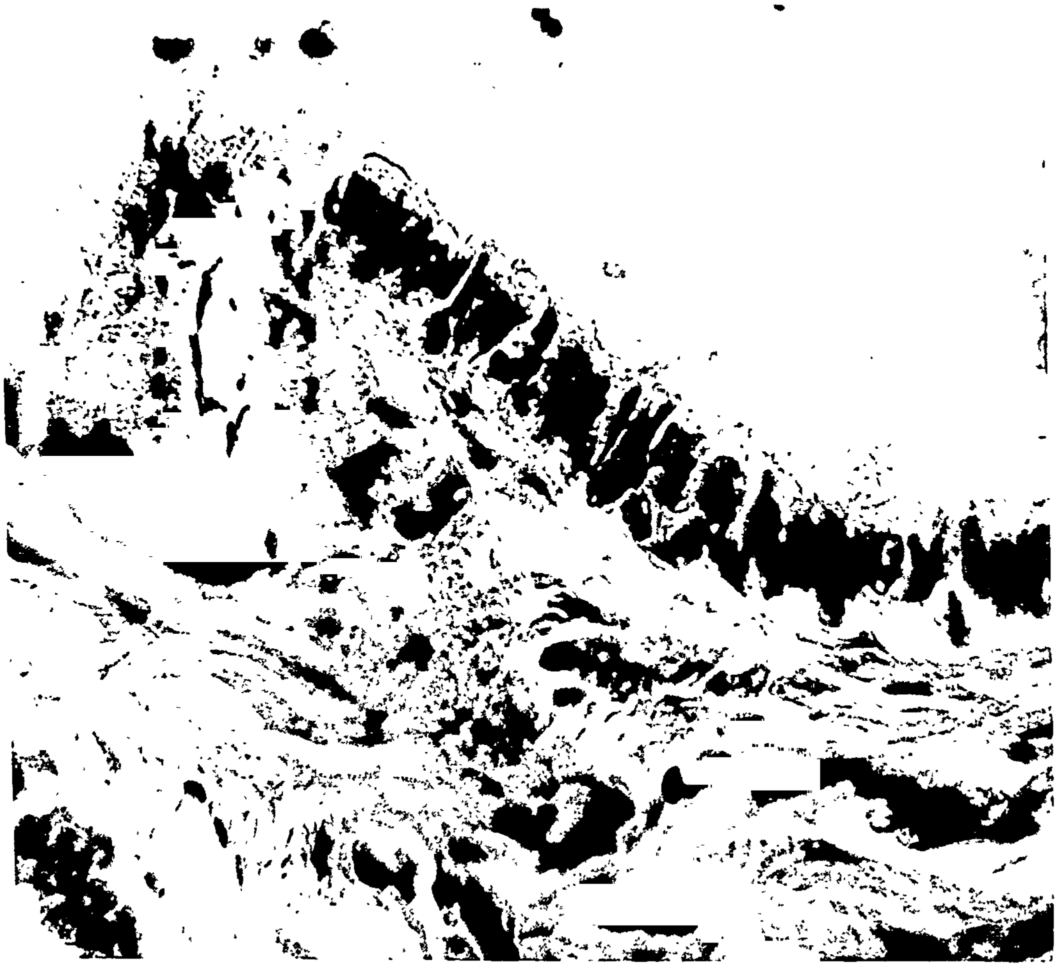

Sachs and Angrist Congenital Cyst of the Myocardium

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CHEMOTACTIC PROPERTIES OF BRUCELLA SUIS

A Study of Phagocytosis of Brucella in Vitro by Normal, Nonimmune Human Leukocytes *

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Although human brucellosis has been subjected to widespread and comprehensive clinical investigations, the details of the pathogenesis of the disease are little understood. Sharp,[1] in a recent summary of reports of investigations of this disease, makes this statement: "A more or less definite type of infectious granuloma is suggested, the most striking feature of which is a nodular lesion resembling the tubercle." From our own observations on the experimental disease [2-6] we are able to confirm Sharp's view in regard to the granulomatous nature of the basic pathological process in brucellosis, but we have not been impressed by any striking resemblance of the lesions to those of tuberculosis. On the other hand, from a review of the very few carefully studied fatal cases of brucellosis that are available, we have been impressed by a resemblance of the lesions to those of Hodgkin's disease.[7] That the latter resemblance may have some particular significance has been suggested by the observations recently reported from this laboratory [3,7-9] that brucella can be isolated from cases of Hodgkin's disease with impressive frequency. It is with the hope of learning more of the pathogenesis of brucellosis and thereby studying further the possible relationship between this disease and Hodgkin's disease that the following investigation of the influence of brucella over leukocytes from the peripheral blood was undertaken.

One of the first things that must be done in the study of the pathogenesis of any infectious disease obviously is to determine what reaction occurs immediately following the introduction of the organism concerned into the tissues. As a general proposition, we know that an immediate local accumulation of phagocytic cells is usually the first

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thing that takes place. The general subject of phagocytosis in relation to infection and immunity has been of great interest since the work of Metchnikoff.[10] He thought that the polymorphonuclear neutrophils were the chief, if not the sole, agents responsible for resistance to bacterial invasion and that the failure of these elements to perform their phagocytic function would result in destruction of the host by the invader. Subsequently other workers [11-14] have adopted a somewhat different view, pointing to the fact that the circulating monocytes and especially the tissue macrophages appear to be the principal defenders of the body. Freedlander and Toomey,[14] for example, have shown that subcutaneously injected staphylococci are phagocytized by both types of cells, but are destroyed chiefly by macrophages. Likewise, Lucké, Strumia, Mudd, McCutcheon and Mudd [15] found that the degree of phagocytosis *in vitro* by the macrophages (including the monocytes) is approximately the same as that of the polymorphonuclear neutrophils. Furthermore, they and others have shown that while, in the experimental animal, the initial response to certain organisms (for example, the tubercle bacillus) is nearly the same for the cells of both types, in the later stages of development of the initial lesion, the monocytes and the macrophages always play the more important rôle.

If one takes into proper consideration these and other well known examples of the development of the initial cellular response to invading bacteria, it may be suspected that the characteristic features of brucellosis may depend to a great extent on the manner in which the phagocytic elements of the body react to brucella. A study of the phagocytosis of brucella *in vitro* by the white cells of the peripheral blood therefore appeared to us to be a logical point of departure for the study of this problem.

Experiments

Experimental Materials. In these experiments, Huddleson's strain 47 of *Brucella suis* from a 48-hour blood agar slant culture was employed; a nonhemolytic *Staphylococcus aureus* cultured in the same manner was chosen for comparative study. Whole human blood obtained by finger puncture with a capillary pipette was used as a source of leukocytes. For supravital studies, preparations were made with neutral red, pinacyanole and a combination of these two dyes (10 drops of neutral red and/or 10 drops of pinacyanole stock solution in 10 cc. of absolute alcohol) spread and dried in a film on the slide. Fixed preparations were stained with the following dyes: Wright's stain, hematoxylin and eosin, Kingsley's stain [16] and Giemsa's stain. Preparations for study were made according to slide-coverslip and hanging-drop methods as described below.

Experiment 1. Supravital Studies of Phagocytosis

A small amount of a saline suspension of live brucella was spread on a slide and upon this a small drop of blood was quickly placed; the preparation was then covered with a coverslip and sealed with a paraffin-vaseline mixture. Similar preparations were made using the staphylococcus. These preparations, on a warm stage in an incubator of 37° C., were observed for as long as 72 hours. Preparations supravitally stained with neutral red and/or pinacyanole also were made according to this technic. The leukocytes proved to be well differentiated in these preparations. The staphylococcus was readily identified both within and outside the cells, but brucella could not be identified except when the organisms were clumped; this was due to the small size of the organisms and to the confusion produced by particles in the plasma or by granules normally present in the cells. Smears of these same preparations were made by sliding the coverslip quickly off the slide; these were allowed to dry, after which the paraffin-vaseline was removed with xylene. These smears were stained according to a variety of methods as indicated above.

Experiment 2. Phagocytosis in Hanging-drop Preparations

Hanging-drop preparations for the study of phagocytosis of killed organisms were made in the following manner: A minute drop of distilled water suspension of organisms was placed in the center of a coverslip, allowed to dry and then gently heated for just a few seconds over a flame. This technic resulted in the production of a small ring of dead organisms on the coverslip. A drop of fresh whole blood was then allowed to clot over the organisms on the coverslip; following this, the coverslip was inverted over a hollow slide and sealed with paraffin-vaseline mixture. Preparations made in this manner were placed in the incubator and observed during periods varying from ½ to 72 hours. Because of difficulty in illuminating the central area of the hanging-drop of blood, observations on these preparations were restricted to the edge of the drop, quite beyond the ring formed by the concentration of organisms. This proved to be an unsatisfactory preparation and it was decided to apply Berman's method,[17] which consists of stripping the clotted drop away from the coverslip with filter paper and fixing and staining the remaining cells. Unfortunately, this usually produced a serious disturbance in the original arrangement of the cells. In further experiments it was determined that satisfactory preparations for study of the cells after incubation could be made by allowing the drop of blood to dry completely in the air, rinsing the dried blood with distilled water to dissolve the hemoglobin, and then drying again in the air. In

preparations of this type, the cells are killed quickly, are not disturbed in their arrangement and, when stained, are easily seen. Numerous stains were applied to these preparations, including Wright's, hematoxylin and eosin, and Kingsley's.[16] Brucella was not well stained by any of these; the staphylococcus stained very well with Kingsley's stain. The Giemsa staining method used by Wolbach in the study of *Rickettsia prowazeki*[18] was employed with excellent results.

Hanging-drop preparations for the study of phagocytosis of living organisms were made as just described except for omission of heating the dried suspension of organisms on the coverslip. (Drying for a short time does not kill all the organisms, as shown by cultures made from the coverslips.)

Control Preparations

Controls for the slide-coverslip method were made, using the same technic and vital stains but with no organisms; these were observed concurrently with the preparation of brucella and staphylococcus.

Controls for the hanging-drop method were made by touching sterile blood agar slants with the loop in the same manner as in making the suspension of organisms and duplicating each succeeding step.

Control of the phagocytic ability of the white blood cells of the blood employed under the experimental conditions was obtained through observation of the action of the whole blood cells toward the staphylococcus used for comparative study.

Experimental Observations

Living white blood cells were studied by the slide-coverslip method, both with and without supravital stains. In many of these preparations there were areas containing clumps of organisms and fluid, but no red cells. In a short time, these areas were invaded by polymorphonuclear neutrophils which rapidly cut swathes through the clumps of organisms, reaching out their pseudopodia and ingesting all of the organisms with which they came in contact. These pseudopodia conformed in shape exactly to the shape of the clumps of organisms and left no organisms behind when they withdrew. This process was observed to continue until the leukocytes were completely filled with organisms.

In the supravitally stained preparations, the staphylococci were seen clearly as unstained refractile bodies inside reddish stained vacuoles in the cytoplasm. The vacuoles were seen to increase in size, but the color became less intense as the cells gradually lost their vitality; after the cells died the vacuoles became colorless. The same type of vacuolization was seen in the preparation made with brucella following phagocytosis of these organisms, but the organisms could not be identified

inside the cells. In the control preparations without organisms this vacuolization began much later and was not nearly so massive. Neither staphylococcus nor brucella, dead or alive, regardless of their location within or outside the cells, was stained by the vital dyes. The organisms were not observed to grow in these preparations, perhaps because of exclusion of the air. No phagocytosis by cells other than the polymorphonuclear leukocytes was seen in these preparations. These cells were active for only a few hours; in contrast, in the unstained preparations they were active for a maximum of about 20 hours, after which they degenerated. In the unstained preparations, after about 20 hours the monocytes and lymphocytes exhibited active motion, which lasted as long as 60 to 70 hours. Even in the active state, these cells did not phagocytize either organisms or other cells. In the supravital preparations, death of these cells occurred within about the same time as that of the polymorphonuclear leukocytes.

In preparations supravitally stained with pinacyanole, phagocytosis by polymorphonuclear cells could be followed with ease. When cells are stained with this dye, the nuclei are dark purple and the cytoplasm light purple; mitochondria are also stained, as are some of the granules. The cells advance a clear layer of ectoplasm which contains no granules; from this many short finger-like processes extend. The ectoplasm first flows around an organism much as water flows along a surface and surrounds an object; next, the granular cytoplasm flows over the organism, which then moves with the rest of the streaming granules. Cells of other types move in a similar manner, but they were not seen to ingest the organisms.

In every hanging-drop preparation of either staphylococcus or brucella, dead or alive, observed from ½ hour incubation upward (with the exception of those in which the organisms were heated beyond a certain point), the neutrophilic polymorphonuclear cells in great numbers migrated to the ring of organisms on the coverslip (Fig. 1) and phagocytized numerous organisms (Fig. 2). The original distribution of the other white cells was not changed. In a few monocytes and eosinophils there were seen one or two organisms (these may have been superimposed), but certainly no marked phagocytosis by these cells was seen in any preparation. Control preparations made without organisms but with drops of distilled water which had been brought into contact with sterile blood agar showed no migration of cells.

When the organisms on the coverslips in the hanging-drop preparations were heated longer than a few seconds (not charred) they did not attract any polymorphonuclear leukocytes, were not phagocytized, and did not stain as they did in other preparations. Hanging-drop preparations made by drying without heating the organisms on the coverslip

showed, after incubation, that the organisms were still alive and had multiplied. In 12 hours, the polymorphonuclear leukocytes at the extreme periphery of the ring of organisms were completely filled with bacteria and some were surrounded by a small colony of organisms (Fig. 3). Comparing these preparations with the gently heated preparations it seemed that the organisms ingested by the polymorphonuclear cells had multiplied within these cells. (It was shown in the slide-coverslip preparations that the cells with ingested organisms are able to live as iong as 20 hours.)

Comment

From the studies reported here, it appears that brucella offers little attraction *in vitro* for any of the normal circulating white blood cells except the neutrophilic polymorphonuclear leukocytes. This observation appears to be out of harmony with the general principle relating to phagocytosis as stated by Lucké and his associates,[15] namely, that "phagocytosis promoting properties of tropins apply similarly to macrophages and to polymorphonuclear leukocytes" and that "the mechanism of bacteriotropin action is the same for both kinds of cells." An explanation of this failure of brucella to attract normal monocytes is highly desirable in view of the fact that observations on the phagocytic activity of the tissue wandering cells—the tissue macrophages or clasmatocytes—in experimental brucellosis [3,5,19] have shown that these cells are commonly engorged with organisms. These latter observations are so impressive that it is suggested that brucella, at least, may propagate best in an intracellular environment. This apparent difference between the action of clasmatocytes and monocytes toward brucella may be a significant one, possibly indicating a fundamental difference between these cells. Such a distinction has been emphasized by Sabin and Doan [20] in their studies of the rôles played by the monocytes and the clasmatocytes in the development of the lesion in experimental tuberculosis. These workers suggest that phagocytosis is carried out chiefly by the clasmatocytes, whereas the monocytes form the tubercle in response to the irritating residue originating in the digestion of the tubercle bacilli by the clasmatocytes. (It is not illogical to postulate the operation of some such mechanism in the development of the brucella lesion and that this may lead to the development of cytological reactions such as those which characterize Hodgkin's disease.[21]) The problem obviously requires further study since in these preliminary experiments there are numerous factors as yet uncontrolled. The artificial conditions of the experiment doubtless were of some importance; this is suggested by the failure of the

staphylococci to attract the monocytes. Perhaps the properties of the particular strains of organisms used also may have been determining factors. Lastly, surely the concentration in the drop of blood of those immune bodies that usually influence the phagocytic activity of the leukocytes was material to the outcome of the experiments. A study of these and other factors concerned are problems for future investigation.

SUMMARY

1. The behavior of white blood cells from the circulating blood of a normal nonimmune person toward *Brucella suis* (Huddleson's strain 47) was studied *in vitro* in slide-coverslip and hanging-drop preparations using fixed and supravital staining technics.

2. In the above described preparations, *Br. suis* provoked an immediate response on the part of the neutrophilic polymorphonuclear leukocytes and was phagocytized quickly by these cells; this response was not exhibited by any of the other leukocytes.

3. When ingested by a polymorphonuclear leukocyte, brucella was found to be surrounded by a vacuole. The ingested organism was not necessarily killed by the cell; in some instances it appeared to multiply within the cell body.

4. Brucella loses some of its staining properties and its chemotactic effects upon the polymorphonuclear leukocytes when it is heated beyond a certain point.

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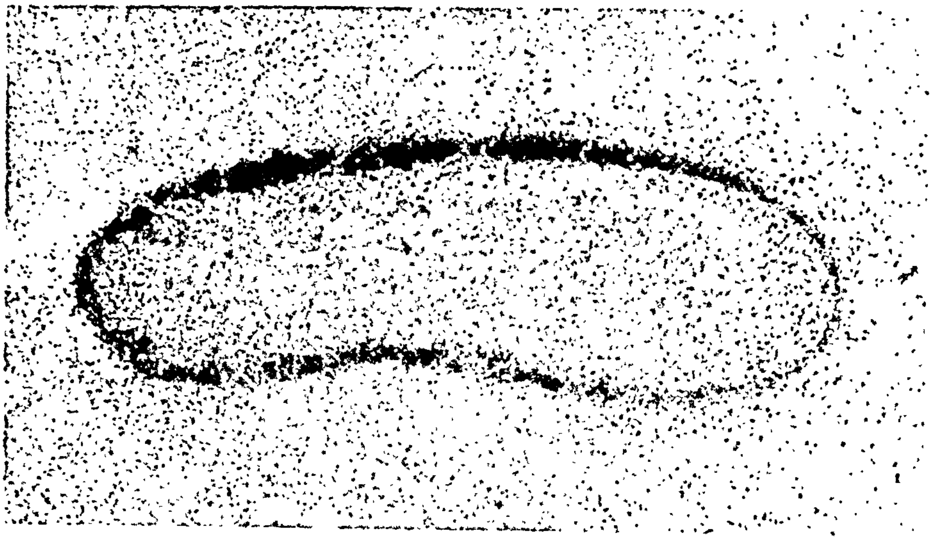
DESCRIPTION OF PLATE

PLATE 32

FIG. 1. Hanging-drop preparation of brucella and nonimmune human whole blood, showing a ring consisting of polymorphonuclear neutrophils which have migrated to and ingested large numbers of organisms previously placed on the coverslip and killed by heating gently. Incubated 1 hour. Giemsa's stain. About × 40.

FIG. 2. A small segment of the periphery of the ring illustrated in Figure 1. The black nuclei of the polymorphonuclear neutrophils are shown, surrounded by cytoplasm packed with large numbers of organisms. The edge of the ring of cells is sharply defined; this outlines exactly the area containing organisms. About × 1100.

FIG. 3. A preparation similar to that of Figure 1, except that the organisms, brucella, were not heated and therefore many were not killed. Incubated 12 hours. The dark areas are masses of organisms which have grown during the incubation of the preparation. The arrow points to a colony of organisms surrounding an engorged polymorphonuclear neutrophil which has migrated from the ring carrying organisms with it. About × 40.



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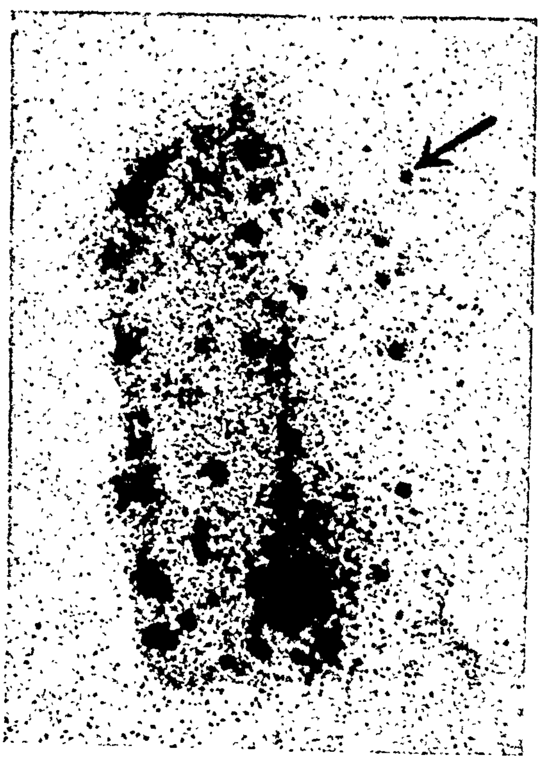

3

Chemotactic Properties of *Brucella suis*

THE REACTION OF THE RETICULO-ENDOTHELIAL SYSTEM IN EXPERIMENTAL AND NATURALLY ACQUIRED BRUCELLOSIS OF SWINE *

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In 1939, Parsons and Poston [1] reported that brucella may be obtained from an impressively high percentage of persons suffering from Hodgkin's disease. In this same report it was noted that it may be quite difficult to differentiate sharply between Hodgkin's disease and brucellosis either by clinical or by pathological-anatomical methods. The work of Parsons and Poston was followed by a clinical study of the relation between brucella infection and Hodgkin's disease by Wise and Poston,[2] whose work seemed to confirm the observations of Parsons and Poston. These investigations indicated a need for a critical study of the suggested etiological relation of brucella to Hodgkin's disease. Before studies of this type could be considered pertinent, however, it obviously was necessary to know something of the pathogenicity of the organisms that had been obtained from the cases of Hodgkin's disease. Forbus and Gunter [3] investigated this phase of the problem, and showed clearly that the strains of brucella recovered from the cases of Hodgkin's disease were capable of producing typical brucellosis in the guinea-pig; the disease produced in this animal, however, appeared to be distinctly less severe than that produced by organisms derived from naturally infected swine. All of these studies emphasized the need for a careful study of the reaction of the tissues to organisms of the brucella group.

It appeared most promising to approach the problems presented by such a study through an investigation of the reactions of the tissues of a variety of experimental animals to different strains of brucella. Accordingly, we have carried out a series of experiments involving the study of brucellosis in both natural and unnatural hosts; the animals employed were swine, dogs, monkeys, guinea-pigs, rabbits and chickens. A summary of our studies in swine and in dogs in combination with a brief note on the more comprehensive problem of the relation between Hodgkin's disease and brucella infection was published in the Proceedings of The American Association of Pathologists and Bacteriologists of 1942.[4] Subsequently, a detailed report of the bacteriological observations in our experiments on swine and dogs was published by Kerby, Brown, Margolis and Forbus.[5]

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In this paper, our purpose is to record our observations on the reaction of the tissues of swine, both in brucellosis experimentally produced in our own laboratory, and in the naturally acquired disease as observed in a group of animals, tissues from which were obtained through the pathological laboratories of the United States Bureau of Animal Industry. The ultimate objective in these studies was to produce a disease which might be considered similar to, if not identical with, Hodgkin's disease in the human being.

Experimental Brucellosis in Swine

Almost without exception the experimental study of brucellosis in swine has been directed along bacteriological, immunological and epidemiological lines. In none of the studies that are available to us has particular attention been devoted to the pathological anatomy of the experimental infection. It is noteworthy that the few adequate descriptions of the experimentally produced lesions relate to observations made on the testis and related structures. Little or no attention seems to have been paid to the reticulo-endothelial system, in which it is generally known that the lesions in naturally acquired infections are commonly found. In the experiments recorded below, complete autopsies were always done, and special attention was directed to the reticulo-endothelial system.

EXPERIMENTS

Since our objectives could be obtained best through a study of prolonged chronic rather than rapidly terminating infection, inoculations were made with a sufficiently small number of organisms and at sufficiently infrequent intervals to assure infection that could be followed over a period of many months. A group of eight pigs, 8 weeks old, newly weaned by a sow from a farm on which no cases of brucellosis had ever occurred, was selected for the experiment. The sow and all of the pigs were studied bacteriologically and immunologically and found to be free of brucella infection. Two of the pigs (VII and VIII) were retained as normal controls for 207 and 205 days respectively and for 240 and 242 days respectively as infected controls. These animals were kept in separate quarters removed from all contact with the experimental group during the normal control period. The six experimental animals were kept in cages in the laboratory, the cages being just large enough to accommodate the animals at maturity. The control animals were kept under similar conditions. Both experimental and control animals were maintained on an adequate diet throughout the experiment.

Strains of *Brucella suis* designated respectively as "Brody" and "ABF 36" were selected for the inoculations. Strain "Brody" was obtained at autopsy from a case of Hodgkin's disease. Its virulence for guinea-pigs had been established.[3] Strain "ABF 36" was obtained from the spleen of a naturally infected hog (Fig. 14); this strain had been shown to be highly virulent for guinea-pigs.

Repeated inoculations of the six test animals were made at intervals of from 7 to 21 days. The inoculum was freshly prepared on each occasion from a 48-hour agar slant culture; it consisted of a saline bacterial suspension standardized by means of the photron-reflectometer. The number of organisms inoculated at one time (sometimes intraperitoneally, sometimes intravenously) varied, beginning with 70 million and increasing gradually to 30 billion. The maximum number of inoculations given to any animal was 32, and the minimum number, 6. Seven oral doses of 30 billion organisms each were administered to one of the normal controls, hog VII, beginning on the 205th day of the experiment, to provide infected controls. Repeated blood cultures were made during the experiment, and numerous cultures were made from the tissues at autopsy.[5] The agglutination titer of the blood for *Brucella abortus* 456 was determined at the time of each culture. The longest period of infection in any of the animals was 447 days, and the shortest period, 98 days. Table I summarizes the important experimental data. Sample protocols in summary are as follows:

Hog I. Summary of Protocol

When the experiment was begun, on September 18, 1940, hog I, a male, 2 months old, weighed 15 lbs. It was fed on "Pig Grower," a commercial feed, supplemented with skimmed milk daily and cabbage every other day. Preliminary blood studies showed: opsonocytophagic index, 8.5; agglutination for brucella 456, negative; blood culture, negative. The animal was killed 245 days after the first inoculation.

Hog I received 29 injections of brucella (Brody strain) beginning with 70 million and increasing rapidly to 30 billion organisms; the first 15 injections were intraperitoneal, the last 14 injections of 30 billion each were intravenous. Except for 3 or 4 injections all were given at intervals of about 1 week.

Temperatures taken every 3 days for the first 2 months ranged between 38.1° and 39.6° C. Opsonocytophagic index, initially 8.5, gradually rose to 46 on the day of death. Agglutination of brucella 456, initially negative, fluctuated widely from 1:2560 to 1:10240. Organisms were never recovered from the blood during the period of intraperitoneal inoculation. Ten colonies of brucella per cc. of blood were recovered 1 week after the first intravenous inoculation. Brucella was recovered 12 times from the blood thereafter.

During the whole course of the experiment this pig showed no symptoms or signs of disease, except for some lassitude for from 24 to 30 hours following intravenous inoculations.

Hog was killed by air embolism and immediately autopsied.

TABLE I

Experimental Brucellosis in Hogs: Summary of Experimental Data

Hog	Organism; Inoculations	Total days	Blood cultures and agg. titer	Autopsy cultures: Lymph nodes	Spleen	Kidneys	Liver	Lungs	Tes-ticle	Peri-toneum	Remarks
I	Brody; 15 i.p., then 14 i.v.	245/10*	21/12† 0/10,240‡	+	+	—	+	+	—	—	No clinical disease; positive blood culture at autopsy; no positive blood cultures during period of i.p. inoculation
II	Brody; 15 i.p., then 17 i.v.	436/117	24/9 0/10,240	—	—	—	—	o	—	o	No clinical disease
III	Brody; 14 i.p., then 17 i.v.	411/92	24/7 0/10,240	—	—	—	—	o	—	o	No clinical disease
IV	Brody; 14 i.p., then 18 i.v.	424/105	24/7 0/10,240	+	—	—	—	o	o	o	No clinical disease
V	Brody; 13 i.p., then 18 i.v.	447/128	23/9 0/10,240	—	—	—	—	o	—	o	No clinical disease; biopsy of spleen, liver, mesenteric node after 12 i.p. inoculations on 85th day; brucella recovered from spleen
VI	Brody; 14 i.p.	98/7	5/0 0/10,240	—	—	o	—	o	o	—	Hog died following weakness and anorexia of 33 days; no fever
VII Normal control, 207 days	ABF 36; 7 oral; infected control	240/129 Inf. control days	6/0 0/5,120	—	—	—	—	o	o	o	No clinical disease; cultures of blood always negative
VIII Normal control, 205 days	ABF 36; 6 i.v.; infected control	242/131 Inf. control days	6/2 0/10,240	+	—	—	—	o	+	o	Acute orchitis, right, 158 days before death; not completely healed at death

i.p. = intraperitoneal; i.v. = intravenous; — = negative; + = positive; o = no cultures.

* Numerator represents total duration of experiment and denominator the number of days between last inoculation and death.

† Numerator represents total number of cultures and denominator the number of positive cultures.

‡ Numerator represents initial agglutination titer for *Brucella abortus* (456); denominator the average titer following inoculations.

Hog V. Summary of Protocol

When the experiment was begun, on September 18, 1940, hog V, a male, 2 months old, weighed 15 lbs. It was fed as was hog I. Preliminary blood studies showed: opsonocytophagic index, 3.5; agglutination for brucella 456, negative; blood culture, negative. The animal was killed 447 days after the first inoculation.

Hog V received 31 injections of brucella (Brody strain), beginning with a dose of 70 million which was rapidly increased to 30 billion. The first 13 injections were intraperitoneal; the last 18 injections, of 30 billion organisms each, were given intravenously at weekly intervals.

The temperature was normal during the first 2 months, and not recorded thereafter. Opsonocytophagic index, initially 3.5, remained unchanged until intravenous inoculations were given; thereafter it varied widely, reaching 53 at the end of the experiment. Brucella 456 agglutination ranged between 1:1280 and 1:2560 during intraperitoneal inoculation; it rapidly rose to 1:10240 after intravenous inoculations were begun.

On the 85th day of the experiment, 8 days after the last of 12 intraperitoneal injections, specimens from the spleen, liver and mesenteric nodes were obtained for biopsy. Cultures of bile, peritoneal fluid and the above-mentioned tissues yielded brucella only from the spleen; paratyphoid bacillus was recovered from the nodes. The animal recovered from the operation and lived without clinical evidences of disease for 362 days.

The animal was killed by air embolism and an immediate autopsy was done.

Hog VI. Summary of Protocol

When the experiment was begun, on September 18, 1940, hog VI, a male, 2 months of age, weighed 15 lbs. It was fed as was hog I. Preliminary blood studies showed: opsonocytophagic index, 7; agglutination for brucella 456, negative; blood culture, negative. The animal died 98 days after the first inoculation.

Hog VI received 14 weekly intraperitoneal inoculations of brucella (Brody strain) beginning with 70 million and increasing to 30 billion organisms. The last inoculation, on December 20, 1940, was made 7 days before death.

The temperature ranged between 38° and 39° C. The opsonocytophagic index, initially 7.0, rose to 14.5, but fluctuated as a rule between 4.5 and 11. Agglutination, initially negative, rose to 1:10240 1 week before death. Blood cultures were never positive.

Thirty-three days before death this hog developed weakness of the hind legs and had bouts of quivering which soon subsided. In spite of eating well for a while, it gradually became weaker and refused to stand and eat during its last few days. During this time no elevation of temperature was noted. The animal was found dead, having died 2 or 3 hours earlier; immediate autopsy was then done. Postmortem cultures were made from the spleen, liver, mesenteric nodes, retroperitoneal nodes, axillary nodes, heart's blood and peritoneal cavity and only postmortem invaders were found.

Hog VIII. Summary of Protocol

When the experiment was begun, on September 18, 1940, hog VIII, a male, 2 months old, weighed 15 lbs. It was fed as was hog I. This animal was used as a normal control for 205 days; after this period it was inoculated with a control strain of brucella (ABF 36) which had been recovered from a naturally infected hog. Preliminary blood studies showed: after normal control period, blood culture

was negative; opsonocytophagic index, 17.5; agglutination for brucella 456, 1:160. The animal was killed 242 days after the first inoculation.

Hog VIII received 6 intravenous inoculations of brucella (strain ABF 36), each containing 30 billion organisms, from April 17, 1941 to August 16, 1941, the control infection period. The opsonocytophagic index rose from 17.5 to 31 but varied greatly. Brucella 456 agglutination, initially 1:160, rose to 1:10240 near the end of the period of infection. Brucella was recovered twice from the blood, the first time 1 week after injection.

One hundred and fifty-eight days before the animal was killed, it was noted that the right testis was swollen and tender. This continued until the testis reached a size one and one-half times that of the other on the 41st day of the disease; after this the swelling subsided. The involved testis then became smaller than its fellow and continued so until the animal was killed. No lymph nodes were palpable.

The hog was killed by air embolism; immediate autopsy followed.

Experimental Results

In Tables I, II and III are recorded the results of the experiments. The clinical and bacteriological observations need no descriptive statement, but the pathological-anatomical findings require detailed consideration.

Of the six animals inoculated with the Brody strain of brucella, only one (hog VI) appears to have died of the infection. The remaining five were killed. In these five animals no gross anatomical lesions were observed except in the lymph nodes and in the peritoneum.

Gross Lesions

The gross alterations of the *lymph nodes* consisted of a slight enlargement accompanied by what appeared to be extensive scarring (Fig. 1). In the scarred areas, particularly at the periphery of the nodes, there was pronounced hemorrhagic pigmentation. When sectioned, the surface was somewhat nodular, the little nodules presumably representing persistent lymphoid tissue; each nodule was isolated by a zone of what appeared to be hemorrhagic scar tissue. These changes were particularly prominent in hog I, in which the peripancreatic lymphoid masses were those most conspicuously altered. The lesions were least well developed in hog II. In this animal enlargement of the nodes was minimal, and hemorrhagic scarring was rarely observed.

On the *peritoneum* of each of the five sacrificed animals there were fibrous adhesions. In only one of this group, hog I, was there indication of testicular inflammation, a lesion that was so prominent in hog VIII, described below (Fig. 9). In hog 1, there were fibrous adhesions between the surface of the *tunica vaginalis,* accompanied by scarring of the testicular capsule. In the *testis* proper no changes were seen.

In hog VI, the one animal thought to have died of infection by the Brody strain of brucella, the *spleen* was somewhat enlarged and its

TABLE II

Experimental Brucellosis in Hogs: Gross Anatomical Findings

Hog	Spleen	Lymph nodes	Liver	Lungs	Testes	Miscellaneous lesions
I	Negative	Enlargement, scarring and hemorrhagic pigmentation of mesenteric, cervical, peripancreatic and retroperitoneal nodes	Negative	Negative	Negative	Fibrous adhesions between visceral and parietal laminae of tunica vaginalis testis; other viscera normal
II	Negative	Slight enlargement of cervical, mesenteric and peripancreatic nodes	Negative	Negative	Negative	Fibrous adhesions between loops of small and large intestine; other viscera normal
III	Negative	Slight enlargement of cervical, mesenteric and retroperitoneal nodes	Negative	Negative	Negative	Fibrous adhesions between small and large intestine and parietal peritoneum; other viscera normal
IV	Negative	Slight enlargement of cervical, mesenteric, peripancreatic and retroperitoneal nodes	Negative	Negative	Negative	Fibrous adhesions between peritoneal surfaces; other viscera normal
V	Negative	Slight enlargement of cervical, mesenteric and inguinal nodes	Negative	Negative	Negative	Fibrous peritoneal adhesions; other viscera normal
VI	Slightly enlarged, capsule roughened and dull	Enlarged, indurated cervical, mesenteric, peripancreatic, retroperitoneal and inguinal nodes	Focal thickening of capsule with fine, fibrinous and fibrous adhesions	Patchy congestion or pneumonia	Negative	Numerous fibrinous and fibrous adhesions between peritoneal surfaces, with small, encapsulated abscess-like pockets filled with necrotic material; entire peritoneum dull; other viscera normal
VII	Negative	Slight enlargement of mesenteric, cervical and inguinal nodes	Negative	Negative	Negative	Other viscera normal
VIII	Negative	Enlarged cervical, mesenteric, peripancreatic and inguinal nodes; large node on right spermatic cord, with focal necroses	Negative	Negative	Rt. testicle small, scarred, with partially calcified abscess-like areas of necrosis; similar changes in epididymis	Other viscera normal

capsule considerably thickened. There were incompletely organized adhesions attached to the surface of the spleen. In the *lymph nodes* there were changes quite like those in the other animals infected with the Brody strain. The surface of the *liver* was covered here and there by organizing fibrinous exudate. On the *peritoneal surface* of the intestine were scattered bead-like masses of exudate, which were of a somewhat caseous character (Fig. 8). These proved to be areas of granulomatous inflammation and they were distributed throughout the peritoneal cavity. With the exception of a few hemorrhages in the *heart* muscle, there were no significant changes in any of the other tissues.

Of the two animals inoculated with the ABF 36 strain of brucella, only one, hog VIII (inoculated intravenously), exhibited at autopsy unmistakable evidence of infection. In the other animal, hog VII (inoculated *per os*), nothing was found except a slight enlargement of the mesenteric, cervical and inguinal *lymph nodes*. In these nodes, however, minimal changes like those found in hog I were present. In hog VIII, which had received intravenous inoculations of the ABF 36 strain of brucella, a general enlargement of the lymph nodes was found. This was due chiefly to changes similar to those described in hog I; however, in addition to these changes an entirely different type of lesion was found in some of the nodes lying along the right spermatic cord. In these inguinal nodes there were foci of necrosis like those observed in the little caseous peritoneal nodules found in hog VI. In the right *testis* of hog VIII destructive lesions were found (Fig. 9). This organ was small, extensively scarred and dotted with little caseous foci; some of these had undergone calcification. A considerable amount of normal testicular tissue remained. The capsule of the testis was thickened by scar tissue, but was still intact. The *epididymis* was reduced in size, extensively scarred and, like the testis, spotted with foci of necrosis.

As a *normal control* for the gross alterations of the *lymph nodes* as just described we have used the submaxillary lymph nodes of ten normal hogs. The lymph nodes from the normal controls were approximately the same size as those of the experimental animals. Otherwise, however, there was a sharp difference. In the normal controls, the structure of the lymph node proved to be quite comparable to that of the normal nodes of man (Fig. 4). Comparison of these nodes with those of the experimental animals, especially hog I and hog VIII, left little doubt of the pathological state of the nodes of the experimental animals (Fig. 2). This interpretation was confirmed by the microscopical studies.

Microscopical Observations

The lesions described above require a detailed microscopical description since they have an important bearing upon the basic problem involved in our study. From the accompanying Table III it may be seen that our chief interest lies in the alterations of the lymph nodes and the development of the granulomatous reaction in the peritoneal cavity and in the testis and epididymis.

With the exception of hog VIII, in which orchitis and epididymitis occurred, the histological alteration of the *lymph nodes* in all of the experimental animals proved to be essentially the same. The characteristic lesion may be described as follows: The earliest change recognized was the massive accumulation of wandering cells of many varieties in the peripheral sinuses; most conspicuous were eosinophilic leukocytes, neutrophilic leukocytes, plasma cells, lymphocytes, and large, actively phagocytic, macrophages. In some of the lymph nodes there were great numbers of red blood cells. Occasionally, multinucleated, actively phagocytic, wandering cells were present. In the older lesions, the cellular masses that originally filled the sinuses, particularly those just beneath the capsule, were replaced by a remarkable growth of reticulo-endothelial cells having the appearance of a highly organized tissue (Figs. 2 and 3). In this reticulo-endothelial meshwork, the reticulum of which was demonstrated sharply by silver impregnation (Fig. 6), were many eosinophilic leukocytes, plasma cells, large mononuclear and multinucleated wandering cells and large macrophages (Fig. 5). In the far advanced lesions the leukocytes and other wandering cells were absent, leaving only the dense reticulo-endothelial structure, in which the reticulum fibers predominated. The disappearance of the wandering cells from the reticulo-endothelial areas sometimes was accompanied by the deposition of amorphous, hyaline material in the tissue spaces. Occasionally relatively large areas of necrosis and hyalinization were seen. In no place was anything resembling tubercle formation seen, and the reaction was not that which one would describe as epithelioid. The reticulo-endothelial reaction in these lymph nodes was, therefore, quite different from that which characterizes the response of the lymph nodes of the guinea-pig to brucella.[3] In some of the lymph nodes showing the sinus reticulo-endothelial hyperplasia the reaction extended to, and eventually completely replaced, the adjacent lymphoid tissue, thus converting the nodes into relatively homogeneous masses of newly formed reticulum fibers (Fig. 2). Red blood cells in the process of disintegration and some blood pigment could be seen in the earlier stages of development

TABLE III

Experimental Brucellosis in Hogs: Microscopic Findings

Hog	Lymph nodes	Spleen	Liver	Peritoneum	Miscellaneous lesions
I	Reticulo-endothelial proliferation of a granulomatous character with marked eosinophilia, hemorrhage and reticular scarring	Some thickening and scarring of capsule with a few macrophages	Some thickening of capsule	Fibrous adhesions	Occasional necrotizing granulomatous nodule in lung; organisms stained in retroperitoneal lymph nodes(?)
II	Dense reticular scarring in sinuses; eosinophilia	Fibrous adhesions over capsular surface	Capsular fibrosis	Fibrous adhesions	Organisms stained in peripancreatic nodes(?)
III	Dense reticular scarring obliterating architecture of nodes; eosinophilia	Negative	Capsular fibrosis	Fibrous adhesions	No other lesions
IV	Dense reticular scarring obliterating architecture of nodes; some active reticulo-endothelial proliferation	Negative	Capsular fibrosis	Fibrous adhesions	No other lesions
V	Dense reticular scarring obliterating architecture of nodes	Negative	Capsular fibrosis	Fibrous adhesions	Tissue obtained for biopsy after 12 i.p. injections showed active granulomatous peritonitis; no active lesions at autopsy
VI	Active reticulo-endothelial proliferation with mononuclear cells clogging sinuses; a granulomatous lymphadenitis	Granulomatous reaction involving capsule and splenic pulp just beneath capsule	Granulomatous reaction over capsule with mononuclear infiltration in periportal area, occasional small necrotic granulomatous foci in liver parenchyma	Active granulomatous peritonitis with focal necrotizing granulomata between intestinal loops	Mononuclear infiltration along fibrous tissue septa of epididymis; active granulomata in periadrenal tissue; organisms stained in peritoneum(?) and lymph nodes(?)
VII	Dense reticular scarring in cortical sinuses; eosinophilia and some active mononuclear proliferation	Negative	Negative	Negative	No other lesions
VIII	Dense reticular scarring obliterating much of the architecture of the nodes; active necrotizing granulomatous reaction in right spermatic node	Negative	Negative	Negative	Marked granulomatous involvement of testicle with necrosis and calcification; giant cell formation and scarring

of the lesion. Giant cells of the Langhans type rarely were seen, but multinucleated giant cells of other types were not rare in the earlier stages of development of the lesion (Figs. 5 and 7).

The histological picture just described was that characteristic of the lymph nodes in general. A significant variation of this picture was observed in the inguinal lymph nodes lying along the spermatic cord of hog VIII, in which there were also present an orchitis and epididymitis. In these lymph nodes, in addition to the changes already described, there were foci of necrosis, each focus being surrounded by a granulomatous inflammatory reaction; eosinophilic leukocytes and large multinucleated wandering cells were prominent in this reaction. The lesion in these lymph nodes was quite like that in the corresponding testis and epididymis, and in the peritoneum of hog VI (Fig. 8). Giant cells somewhat resembling the Langhans type occasionally were found near the necrotic foci.

In summary, the changes found in the lymph nodes in our infected animals can be described as those associated with the development of a granulomatous inflammatory reaction. This reaction was of such a character as to result eventually in complete replacement of the lymphoid tissue by a reticulo-endothelial scar (Figs. 2 and 3).

The histological reaction of the *peritoneum* was best illustrated by hogs V and VI. The most revealing tissues from hog V were obtained from the spleen by operation on the 85th day of the experiment, after 12 intraperitoneal inoculations of brucella had been made. The tissues from hog VI were obtained at autopsy on the 98th experimental day, after the administration of 14 intraperitoneal inoculations. (Death in this animal was related specifically to the brucella infection.) The inflammatory reaction of the peritoneum as shown in both of these animals was essentially granulomatous. The cellular exudate was made up predominantly of large mononuclear (sometimes multinucleated) wandering cells, polymorphonuclear leukocytes (eosinophils being especially prominent), plasma cells, lymphocytes, and large macrophages engorged with phagocytosed material. Necrosis of the exudate was found wherever it was abundant (Fig. 8). The lesion showed a profound tendency to heal, as indicated by the proliferation of fibroblasts and blood vessels. That healing eventually was complete was clear from the finding of nothing except fibrous adhesions in the peritoneum in hog V at the autopsy many months after the tissues for study were removed at operation. The discovery of peritoneal fibrous adhesions in all six of the experimental animals that received intraperitoneal inoculations appears to be a fair indication that granulomatous lesions such as those just noted must have developed in all of these

animals. Thus, disease due to brucella actually was established in all of these animals, even though the clinical and bacteriological studies of four of them left some doubt of established infection.

Certain other microscopical findings are of sufficient importance to be included here. One of these was the presence of focal granulomatous lesions in the *liver* of hog VI identical with those seen in typical brucellosis of the guinea-pig (Fig. 7). Accompanying these there was a marked portal round cell inflammatory reaction. The latter also was like that seen in brucellosis of the guinea-pig, and, furthermore, was like that described in hogs spontaneously infected with brucella.

Another lesion of interest was found in the *bones*. This consisted of a replacement of the marrow by edematous connective tissue and fat containing a variety of wandering cells. This, like the liver lesion, was most conspicuous in hog VI. Neither the hepatic lesions nor those in the bone marrow were characteristic of the animals as a group.

Lastly, in the *testis* and *epididymis* of hog VI, there was a fairly diffuse, chronic inflammatory reaction, but there was no lesion comparable to that in hog VIII now to be described.

The histological picture of the lesion in the *testis* and *epididymis* of hog VIII was that which frequently has been described in natural infections of swine and bovines by brucella. The characteristic lesion was an inflammatory reaction in which were mononuclear wandering cells and eosinophilic polymorphonuclear leukocytes in abundance, accompanied by a few neutrophilic leukocytes and a variety of small mononuclear wandering cells. The lesion seemed to develop somewhat along the following lines: The organisms appeared to have localized in the tissue surrounding tubules of the epididymis and the testis. This was followed by the development of the granulomatous reaction just outside the tubular structure and its extension through the lining of the tubules, finally to fill the lumen (Fig. 10). The exudate eventually became necrotic, and, at the same time, the epithelial lining of the tubules was destroyed. Necrosis was followed by deposition of calcium, after which the whole process subsided (Figs. 11 and 12). Complete healing resulted in the formation of a hyaline scar (Fig. 13). As the process healed a variety of giant cells, chiefly of the typical foreign body type, appeared in and about the partially calcified granulomatous lesion. Other types of giant cells were seen occasionally; some of these resembled the large multinucleated giant cells which characterize the Hodgkin's reaction but most of them were more like the Langhans giant cells. Nowhere did we observe typical tubercle formation; in fact, the reaction showed no striking resemblance to that produced by the tubercle bacillus, although the individual components of the reaction

were identical with those of tuberculous lesions. The result of the confluence of many lesions of the type just described was extensive destruction of both the testis and epididymis, reducing the whole to a contracted scar (Fig. 9). *In summary,* the testicular and epididymal lesion was essentially a granuloma which, though bearing a certain resemblance to the granulomatous lesions in a number of other diseases, was actually unlike any one of these; it probably is specific for brucella.

As may be seen in Table I, brucella was recovered at autopsy or at operation from the tissues of four of our eight animals. Nevertheless, in none of the tissues were we able to demonstrate the organisms by histological methods; this was so in spite of the fact that we routinely used for histological study those tissues from which the organisms had been obtained by culture.

Before discussing our observations on the reaction of the tissues of the hog to brucella in the experimentally produced disease, it will be useful to record our findings in a study of the naturally acquired disease in swine.

Naturally Acquired Brucellosis in Swine

Naturally acquired swine brucellosis has been studied clinically, immunologically, and epidemiologically by a great number of workers. The most comprehensive study of the problem is that of Thomsen.[6] Thomsen's work and that of more recent contributors, particularly Giltner [7] and Feldman and Olson,[8] have been summarized in Huddleson's monograph [9] and need not be reviewed here. It should be noted, however, that only a few of the students of this disease in the lower animals, particularly the hog, have devoted any attention to the extragenital lesions produced by brucella. Especially have the lymphoid tissues of the body, with the exception of the spleen, been neglected. Giltner, and Feldman and Olson have made good descriptions of lesions produced by brucella in the bodies of the vertebrae, and Christiansen and Thomsen [10] have made careful histological studies of the lesions in the genital organs in swine. Creech [11] seems to be the only student of the disease who has observed lesions in the lymph nodes.* It appears that naturally acquired brucellosis in swine is a relatively benign infection which only occasionally gives rise to lesions of any importance outside of the genital tract. The more important well known extragenital lesions are swelling of the joints, subcutaneous abscess-like lesions, abscess-like lesions in the bone, particularly the vertebral bodies, and caseous, terminally calcified, focal, granulomatous lesions in the spleen (Figs. 14 and 15). The lesions developing in the

* Creech describes very briefly a severe, caseating type of portal lymphadenitis in one hog.

uterus and responsible for abortion, those in the testis which result in sterility, and those in the bones appear from the descriptions to be essentially alike. They are granulomatous lesions with a tendency to necrosis and sometimes suppuration; complete healing occurs and results in scar formation and, occasionally, calcification.

Through the courtesy of Dr. C. L. Davis of the Denver Laboratory of the Federal Bureau of Animal Industry we have had an opportunity to study the lymph nodes and the spleen from four swine condemned by the meat inspection service because of the presence of lesions in the spleen that generally are thought by that service to be due to infection by brucella. From the lesions in the spleen in three of these animals, *Brucella suis* was obtained either by direct culture or by guinea-pig inoculation.

The lesion in the *spleen* which appears to be characteristic in these four animals is a granulomatous nodule which superficially resembles the tuberculous nodule of firm type commonly seen in man. Some of these nodules are caseous and some are quite hard; the latter consistency is the result of extensive calcification (Fig. 14). Microscopically the splenic nodules show at the periphery a mass of large mononuclear wandering cells, epithelioid cells, eosinophils and a variety of small mononuclear wandering cells. The center and the bulk of the lesion is formed by necrotic exudate, the cells of which are unidentifiable. Langhans giant cells are present, but giant cells of other types, presumably representing greatly enlarged and multinucleated wandering cells of the reticulo-endothelial system, are more common; these appear at the periphery of the lesion. In the old lesions, the necrotic inflammatory exudate is extensively calcified, and the lesion as a whole is surrounded by a zone of fibrous tissue (Fig. 15). The intervening tissues of the spleen are normal. We isolated *Br. suis* from an extensively calcified and encapsulated, but still active, lesion in one of our cases (this organism is that designated "ABF 36" and used in our experimental studies).

In only one of the four hogs with brucella lesions in the spleen were we able to study the lymph nodes, and these nodes showed no gross abnormality. In the peripheral sinuses and along the trabeculae in the interior of the node there was a granulomatous reaction with marked hyperplasia of the reticulo-endothelial elements and an excessive infiltration of eosinophilic leukocytes, an alteration of the lymphoid structure identical with that found in our experimentally infected animals.

From this brief description of our own observations, as well as from the observations of others, it is clear that *Br. suis* is capable of produc-

ing in the spleen a lesion which is essentially like that which it produces in the testis, the epididymis, and in the wall of the placenta of the pregnant sow.[6,10] It may be recalled at this point that in none of our experimental infections did we produce lesions of any type in the spleen.

Discussion

Before commenting on the changes that we have described in the lymph nodes of our inoculated animals, it is first necessary to determine whether or not infection by brucella actually was established in these animals. The details of this matter have been dealt with in a preceding paper,[5] but reference to Table I makes it possible to draw certain general conclusions. The following criteria have been used for determining active infection in our animals: (1) Repeatedly positive blood cultures not earlier than 1 week after inoculation; (2) high agglutination titers for brucella maintained throughout the experiment; (3) recovery of brucella from the tissues at autopsy as long as 131 days following the last inoculation; and (4) the presence of lesions known to be characteristic of brucella infection at autopsy or at exploratory operation. If judgment be based upon all four of these criteria separately or in combination, it seems fair to say that all of our inoculated animals had brucellosis even though only two of the eight presented clinical evidence of disease. In view of the difficulty in recovering brucella from animals known to be infected, and in view of the striking tendency of the lesions of brucellosis to heal, our failure to recover brucella from the tissues of all of our animals at autopsy cannot be considered a serious argument against the existence of infection in these animals.

In the course of our experiments we have found that the hog clears the blood of organisms within a period of from 1 to 3 weeks following inoculation of the blood stream with brucella; nevertheless, in animals in which this has occurred, the organisms can still be isolated from the tissues (lymph nodes) as long as 7 months after the inoculation.

Thus, it seems that brucella has a striking tendency to become isolated in the lymph nodes where it may remain and retain its pathogenic properties for a very long time. Under such conditions a mild but prolonged tissue reaction would be expected; indeed, alterations of the lymph nodes such as we have seen in our animals would be the natural outcome. Thus, it appears that we are entitled to attribute the lesions in the lymph nodes that are common to all of the experimental animals to the action of brucella.

One may ask why lesions in the lymph nodes of the experimental animals occur so regularly in the absence of changes in the spleen, the

bone marrow and other reticulo-endothelial tissues. Furthermore, why should the chronic, nonnecrotizing lesions in the lymph nodes in the experimental animals differ so greatly from the destructive lesions in the testis and the spleen (and occasionally in the lymph nodes) that occur in the naturally infected animals? We have no satisfactory answer to these questions unless it be that the hog is extraordinarily resistant to brucella infection and, therefore, usually suffers no destruction of its tissues, or that the necrotizing lesion is the only type commonly recognized by those who have most contact with these animals. The virulence of the organisms used in our experiments may likewise be an important factor. To this is to be added the significant fact that it is the lymph nodes that harbor the organisms for long periods of time. The literature dealing with swine brucellosis appears to be in harmony with this view of the matter.

Even though it appears permissible for us to interpret the lesions that we have described in the lymph nodes of our experimental animals as due to infection by brucella, this reaction in the lymph nodes cannot be considered peculiar to infection by this organism. The lesion is known to veterinary pathologists, who describe it as "chronic sclerosing lymphadenitis" and attribute it to a great variety of pathogenic agents. None of these agents appears to have been studied critically. Joest [12] accuses a variety of bacteria. From what we now know of brucella infections, not only in swine but in other animals, it may be that this chronic sclerosing lymphadenitis is a common expression of brucellosis in the lower animals.

A few comments may be made on the findings in our experimental animals as they may relate to the question of a possible relationship between brucella infection and human Hodgkin's disease. From the observations that we have made in the hog, it is not possible to say more than that brucella produces in this animal, as it does in other animals, a chronic, granulomatous form of reticulo-endothelial reaction, an inflammation, the essential features of which are those we recognize as belonging also to the basic reaction in human Hodgkin's disease. Thus far we have not been able to produce in swine by means of brucella inoculation a disease entity that can be considered similar to human Hodgkin's disease. That such an accomplishment is beyond possibility is, however, another matter. In fact, we know far too little about brucella infection, not only in swine and in other lower animals but also in man, to exclude such a possibility. From what we have seen in the hog, in the dog, in the rabbit, in the guinea-pig, in monkeys and, indeed, in man, it is clear that brucella has an extraordinary capacity to stimulate the proliferation and alteration of the reticulo-endothelial

system. Hodgkin's disease in man probably represents a reaction of the reticulo-endothelial tissues such as might well be produced by an agent with the pathogenic properties of brucella.

In conclusion, it is worth noting that there does occur in swine a peculiar granulomatous disease of unknown etiology that bears a certain resemblance to human Hodgkin's disease. Through the courtesy of Dr. C. L. Davis of the Federal Bureau of Animal Industry we have had an opportunity to study a group of swine affected with this disease. A report of these studies will be made in a subsequent communication, but it is worth noting here that we have attempted already to show a connection between swine brucellosis and this peculiar Hodgkin's-like swine disease by comparing the experimentally produced and naturally acquired brucellosis described in this paper, with the Hodgkin's-like disease in the group of animals just mentioned. At this writing it appears very difficult, if not impossible, to bring together as representing a single entity what we know to be brucellosis in swine and this Hodgkin's-like swine disease. Thus, the possibility of showing a relationship between swine brucellosis and swine "Hodgkin's disease" seems at this time to be surrounded by difficulties equally as great as those involved in the demonstration of a possible relationship between human brucellosis and human Hodgkin's disease.

Summary and Conclusion

1. A strain of *Brucella suis* isolated from a case of Hodgkin's disease and kept on artificial media for a long period has been shown to be pathogenic for the hog.

2. A strain of *Br. suis* freshly isolated from a naturally infected hog has been shown to be more highly pathogenic for the hog than the strain of *Br. suis* isolated from the case of Hodgkin's disease.

3. Hogs are highly resistant to *Br. suis* infection; they survive repeated large doses of the organisms given either intraperitoneally, intravenously, or by feeding.

4. The site of injury in the hog by *Br. suis* varies with the route of infection, but the reticulo-endothelial system, especially the lymph nodes, appears to be regularly affected.

5. Highly pathogenic *Br. suis* can be recovered from lymph nodes of hogs as long as 131 days after intravenous inoculation and in the absence of clinical disease.

6. The reticulo-endothelial cell with all its potentialities for morphological variation and proliferation is the chief reacting cell in swine brucellosis.

7. The lesions in the lymph nodes of the hog during the early stages

of *Br. suis* infection are characterized by: (a) marked proliferation of the reticulo-endothelial cells of the sinuses, the greatest number of cells developing into mature macrophages; (b) the presence of multinucleated giant cells; (c) marked infiltration of eosinophils; (d) occasional foci of necrosis followed by calcification.

8. The chronic lesions in the lymph nodes caused by *Br. suis* infection in hogs are characterized by a marked proliferation of the reticulo-endothelial cells resulting in replacement of the lymphoid structure by a dense reticular scar in the meshes of which a few eosinophils and mononuclear cells persist.

9. The healing of the necrotizing lesions of swine brucellosis, wherever they occur, is followed by reticulum-like scarring, occasionally with calcification.

10. It has not been possible in these experiments to produce in hogs by prolonged infection a disease entity that is comparable to human Hodgkin's disease; the basic reaction of the reticulo-endothelial tissues of the hog to brucella are, however, quite comparable to the basic reticulo-endothelial reaction that characterizes Hodgkin's disease in man.

11. There occurs in hogs a natural disease of unknown etiology, the lesions of which show a certain resemblance to those of human Hodgkin's disease. The nature of this disease and its possible relation to swine brucellosis requires further investigation.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 33

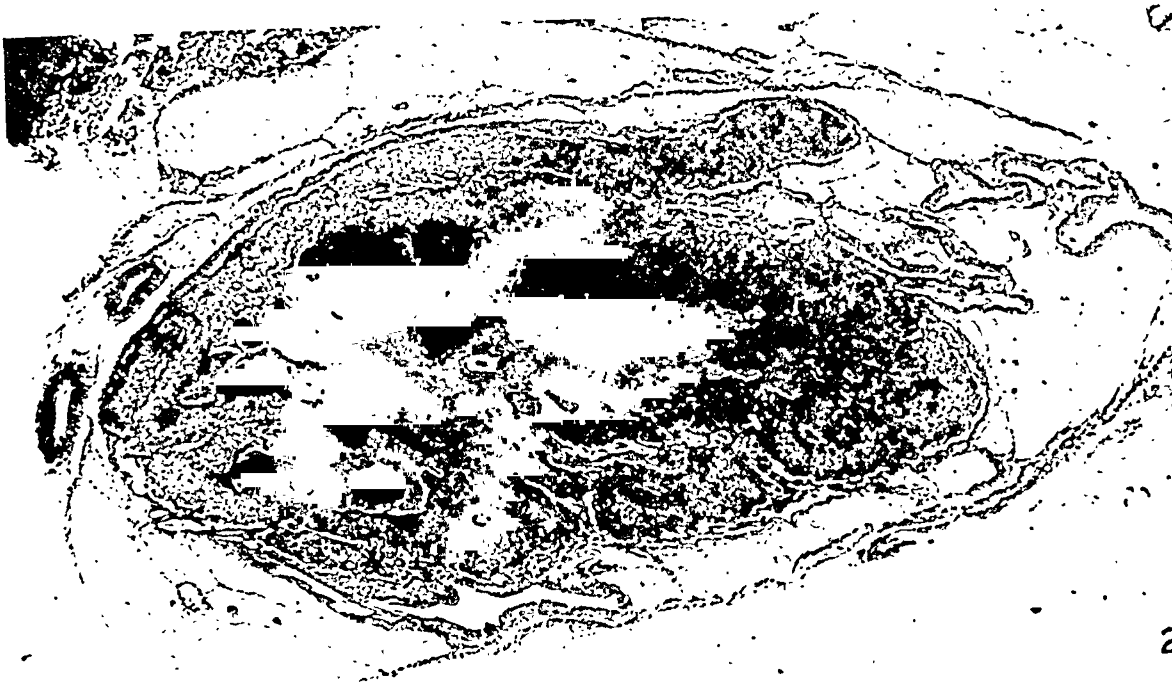
FIG. 1. Experimental brucellosis, hog I. Four lymph nodes, photographed at actual size, from a hog which had received within 245 days 29 inoculations of a strain (Brody) of brucella recovered from a case of Hodgkin's disease. The nodes are not significantly enlarged, but they show the scarring and hemorrhagic pigmentation characteristic of the nodes in all of the animals in this group. The two nodes at the left are seen in section and those at the right are unsectioned.

FIG. 2. Experimental brucellosis, hog II. Whole section of a small lymph node from an animal which had received within 436 days 32 inoculations of a strain (Brody) of brucella obtained from a case of Hodgkin's disease. The gray areas represent the reticulo-endothelial reaction, in the course of which the lymphoid structures are replaced. The black areas represent persistent lymphoid tissue. This may be compared with Figure 4, a photograph of a normal lymph node of a hog made at the same magnification. × 31.

FIG. 3. Experimental brucellosis, hog I. Photomicrograph of a section of one of the lymph nodes shown in Figure 1. The tissue represented, similar to that in the gray areas of Figure 2, shows the granulomatous character of the reaction; no lymphoid tissue, as such, remains. This may be compared with Figure 4, a normal control, and Figure 5, a higher magnification of a similar reaction. × 130.

FIG. 4. Photomicrograph of a section of a normal lymph node from a normal control hog for comparison with the nodes of the experimentally infected hogs as illustrated in Figures 2 and 3.

1



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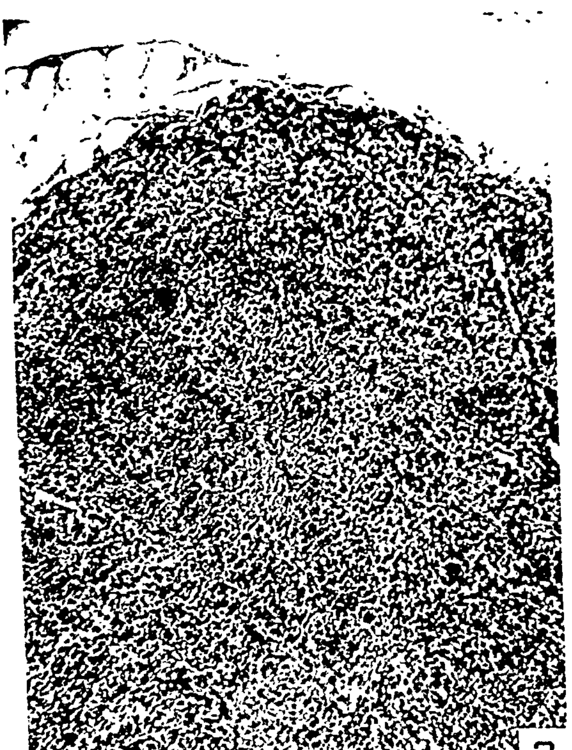

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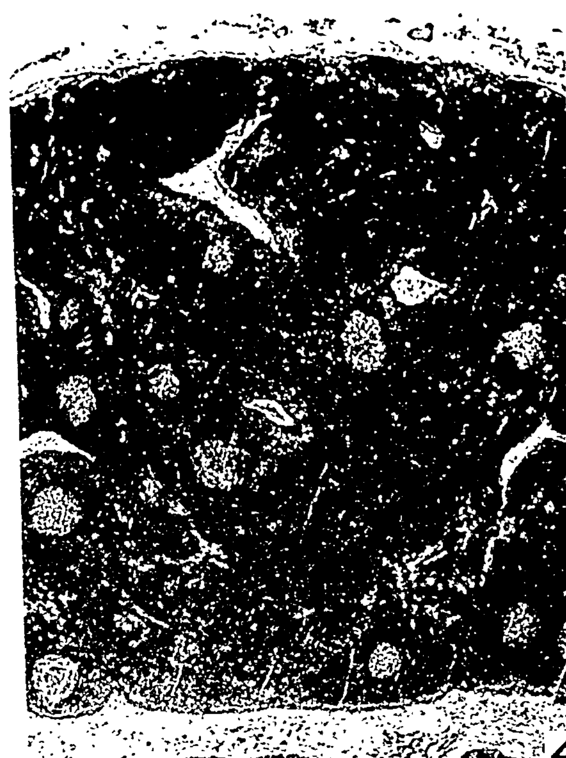

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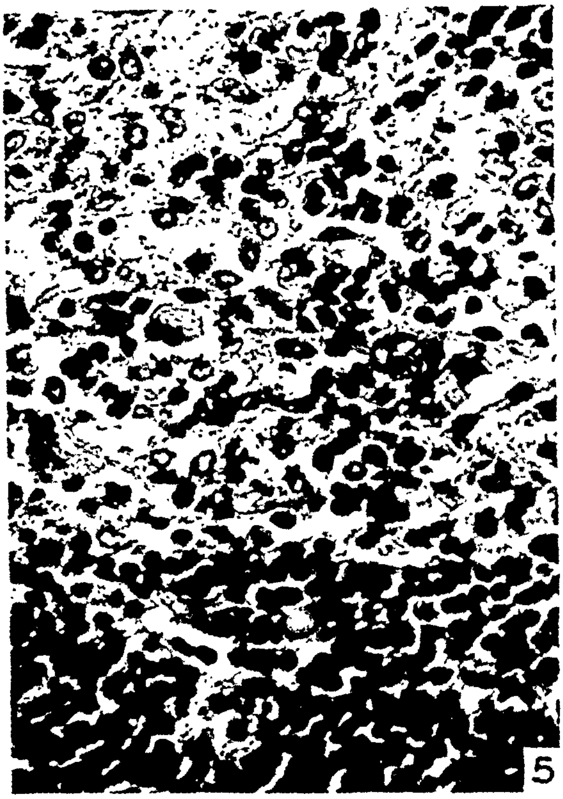
Plate 34

Fig. 5. Experimental brucellosis, hog II. Granulomatous reaction in a lymph node, like that shown in Figures 2 and 3 but at higher magnification. This animal had received 32 inoculations of the Brody strain of brucella within 426 days. × 395.

Fig. 6. Experimental brucellosis, hog II. Section of a lymph node like that in Figure 2, stained to demonstrate the reticulum of the reticulo-endothelial reaction which fills the peripheral sinus and extends to replace the cortical lymphoid tissue. × 245.

Fig. 7. Experimental brucellosis, hog VI. A focal granulomatous reaction in the liver near the portal area of the lobule from the only animal in the group that died of brucellosis. The cells of the reaction are eosinophilic leukocytes, large mononuclear wandering cells, lymphoid cells and multinucleated giant cells. This may be compared with Figure 8, a granulomatous reaction in the peritoneum of the same animal, dead on the 98th day; and with Figure 5, a granulomatous reaction in a lymph node of another infected animal that survived and was killed on the 426th day. × 395.

Fig. 8. Experimental brucellosis, hog VI. A necrotizing granulomatous lesion on the surface of the liver of an animal that died of brucellosis on the 98th day of infection by way of the peritoneum. This may be compared with Figures 5 and 7. × 130.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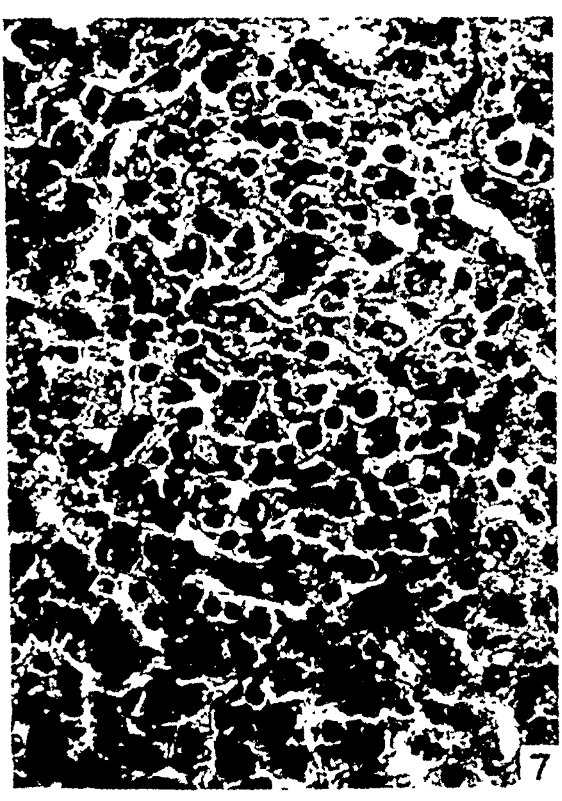
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8

Brown, Forbus and Kerby

Reticulo-endothelium in Brucellosis

PLATE 35

FIG. 9. Experimental brucellosis, hog VIII. Healing granulomatous orchitis and epididymitis from an animal infected for 131 days with a strain (ABF 36) of brucella obtained from a partially calcified granulomatous lesion in the spleen of a naturally infected hog (Fig. 14). The normal left testis is shown for comparison. (The details of development of the characteristic lesions are shown in Figs. 10, 11, 12, and 13.) Two-thirds actual size.

FIG. 10. Experimental brucellosis, hog VIII. The earliest phase in the development of epididymitis and orchitis due to brucella. The lesion shown is a small *granuloma which developed just beneath the epithelium of a tubule of the* epididymis and subsequently destroyed the epithelium, spilling its substance, including the organisms, into the lumen. × 245.

FIG. 11. Experimental brucellosis, hog VIII. Granulomatous orchitis. This is a relatively early stage in the development of the lesion which is found in the supporting tissue of the testis between the seminiferous tubules. As in the epididymis, the lesion extends to and destroys the tubule; the granulomatous tissue finally takes the place of the tubular structure. × 245.

9

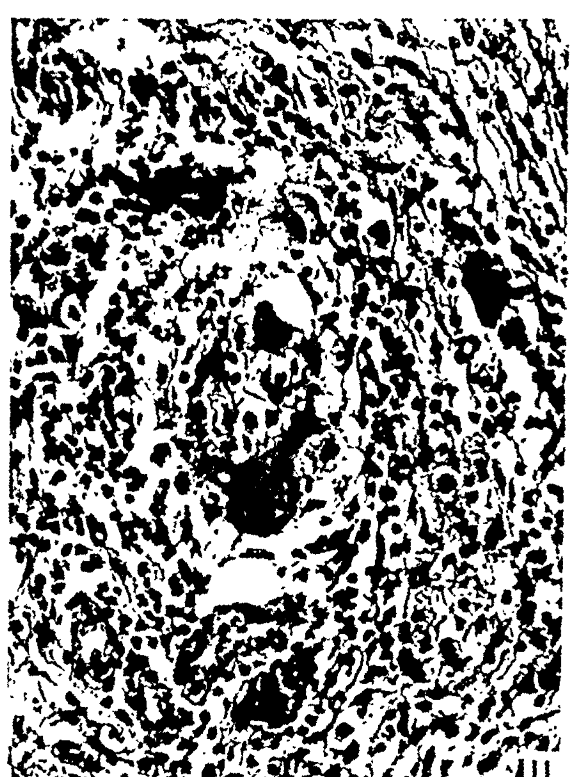


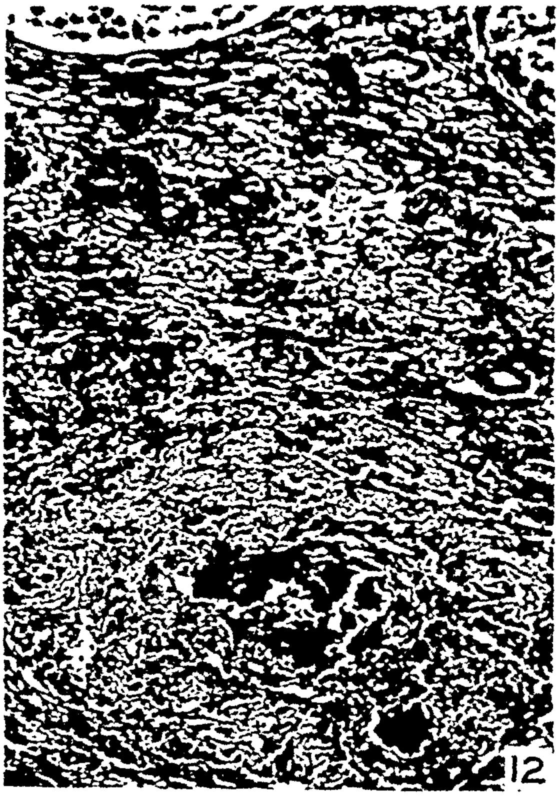
PLATE 36

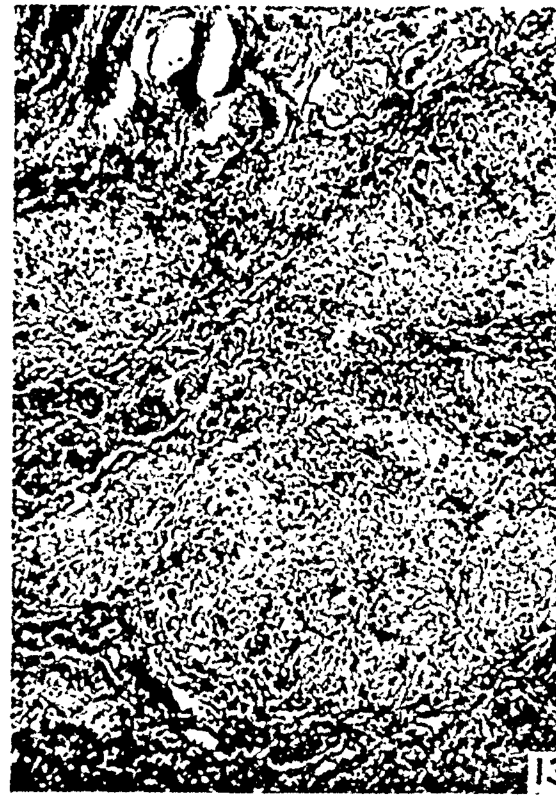
FIG. 12. Experimental brucellosis, hog VIII. A partially decalcified granulomatous lesion characteristic of the healing phase of orchitis due to brucella. × 130.

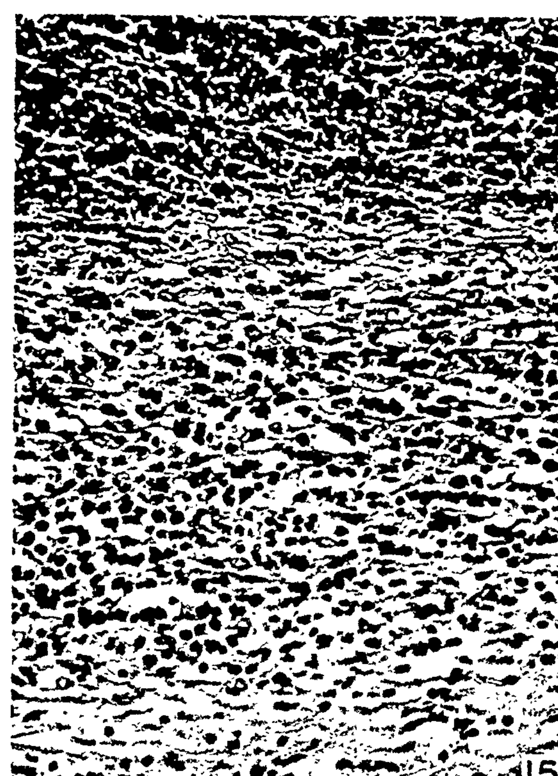
FIG. 13. Experimental brucellosis, hog VIII. Hyaline scars resulting from complete healing of lesions in the testis like that shown in Figures 11 and 12. × 245.

FIG. 14. Naturally acquired brucellosis, hog ABF 28. Two well encapsulated and partly calcified, but still active, granulomatous lesions in the spleen of a hog. The strain of brucella (ABF 36) used in the control infections in hogs VII and VIII was recovered from a lesion like this in another animal. × 27.

FIG. 15. Naturally acquired brucellosis, hog ABF 37. The tissue represented is the inner zone of the capsule of a lesion like that shown in Figure 14. The exudate is composed chiefly of eosinophilic leukocytes and mononuclear cells. In lesions of this type that are not so nearly healed, large mononuclear leukocytes and multinucleated giant cells form the inner border of this zone, next to the centrally situated necrotic mass of cells. The lesion is comparable to that shown in Figure 8. × 245.







Brown, Forbus and Kerby

Reticulo-endothelium in Brucellosis

THE RELATION OF HODGKIN'S DISEASE, LYMPHOSARCOMA AND RETICULUM CELL SARCOMA *

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Although medical literature is replete with publications on Hodgkin's disease, lymphosarcoma and reticulum cell sarcoma, there are only sporadic reports on the relationship of these seemingly distinct entities. Gibbons,[1] in 1906, was one of the first to consider Hodgkin's disease as a variant of lymphosarcoma. Coley [2] shared this view and proposed that the name "Hodgkin's disease" be replaced with "lymphosarcomatosis." In 1913, Oliver [3] concluded that "all constitute a series of neoplastic processes of the lymphatic glands, which differ not so much qualitatively as quantitatively" and that "it is the predominate cell type which allows one to class the tumor as a lymphosarcoma, an endothelioma or Hodgkin's disease." Apparently Mallory [4] was of the same opinion for he considered lymphocytoma, lymphoma, lymphosarcoma, pseudoleukemia, lymphatic leukemia and Hodgkin's disease under the all inclusive term of lymphoblastoma.

A search of the more recent literature discloses only three pertinent case reports since 1928. One was that of lymphatic leukemia associated with reticulum cell sarcoma.[5] Another was a case of lymphoblastoma, Hodgkin's disease and tuberculosis [6] while a third was a case of Hodgkin's disease with sarcomatous features associated with lymphatic leukemia.[7] During this same period, however, general interpretations based upon large series of cases have been somewhat more numerous. Thus, concerning the relationship of Hodgkin's disease and lymphosarcoma, MacCarty [8] stated that they have "a common neoplastic cellular origin"; Levin [9] concluded that they are "phases of the same pathologic entity and the two may exist in the same patient"; Ginsburg [10] remarked that "they are merely variations of the same disease," and Cohn and Richter [11] were of the opinion that they are "genetically related." With reference to the relationship among lymphosarcoma, lymphatic leukemia, Hodgkin's disease and mycosis fungoides, Warthin [12] concluded that "transition forms exist between all of these groups and one type may be transformed into another." Commenting upon the same group of diseases, Sugarbaker and Craver [13] agreed that the borderlines between them "remain rather shadowy at present." Finally, the close relationship between the sarcomatous type of Hodgkin's disease and reticulum cell sarcoma has been clearly expressed by Callender.[14]

* Received for publication, April 22, 1944.

Because of the relative paucity of these reports, we shall consider in this paper a selected group of six cases that at one time were diagnosed as Hodgkin's disease and at another time as lymphosarcoma, and that at autopsy showed various combinations of Hodgkin's disease, lymphosarcoma and reticulum cell sarcoma. In agreement with the above-mentioned observers, we believe that these processes are not only genetically related but that fundamentally they are merely phases of the same disease. This contention is supported both by the cases which follow and by the experimental work of Miller and Turner.[15] The latter will be fully elucidated in the discussion.

Report of Cases

Case 1

In May, 1939, a white woman, 45 years old, was admitted to another hospital complaining of weakness, generalized lymphadenopathy and enlarged spleen of 10 months' duration. Biopsy of a lymph node disclosed Hodgkin's disease. In May, 1941, an axillary node removed at the Jefferson Hospital showed a lymphosarcoma. Following x-ray therapy, her condition improved until September, 1943, when, in addition to the return of the previous signs and symptoms, she developed marked anemia and showed hypoplasia of the sternal bone marrow. From the third lymph node removed at this time, Hodgkin's disease was diagnosed. She died 3 months later.

The axillary node removed on May 29, 1941, measured 1.5 cm. in diameter. Microscopically, only a few follicles were still discernible and they were greatly enlarged, ill defined and in one or more areas blended imperceptibly with the cells of the adjoining parenchyma. Throughout the node most of the cells were large lymphocytes and the remainder were of the small variety (Fig. 1). Mitosis was occasionally seen.

A mass of cervical nodes removed on September 30, 1943, measured 2.5 by 1.5 cm. They were pinkish gray, moderately firm and contained several necrotic foci measuring as much as 3 mm. across. Histological sections disclosed a picture typical of Hodgkin's disease (Fig. 2). There were varying degrees of fibrosis, focal areas of necrosis, and a diffuse infiltration with large and small lymphocytes, plasma cells, a few polymorphonuclear leukocytes, scattered eosinophils and typical Sternberg-Reed cells. There were only a few poorly stained and ill defined reticulum cells in the background.

Autopsy performed on December 14, 1943, showed considerable emaciation and a "scaly" dermatitis. The lymph nodes in the left axilla measured as much as 2 cm. across while those of the mediastinum and along the abdominal aorta measured up to 5 cm. in diameter. They were matted together quite firmly and upon section disclosed pinkish

gray tissue with occasional foci of necrosis and hemorrhage. The liver weighed 1860 gm. and contained many circumscribed nodules measuring as much as 1.5 cm. in diameter. The spleen weighed 550 gm. but showed no tumor nodules. In the bone marrow of the lower thoracic and lumbar vertebrae there were irregular gray foci. Tumor nodules in the left pleura measured 1 to 2 mm. in diameter. The left pleural cavity contained 750 cc. of straw-colored fluid and the right pleural cavity contained 2000 cc. of similar fluid.

Microscopically, the lymph nodes exhibited three distinct pictures. All showed a complete disappearance of the normal architecture. In some there was a slight but uniform increase of reticulum and a diffuse infiltration with mature lymphocytes (Fig. 3). There were no other cells present. In other nodes there were scattered areas of necrosis, marked fibrosis and a cellular infiltration of lymphocytes, plasma cells, polymorphonuclear leukocytes, occasional eosinophils and a few typical Sternberg-Reed cells (Fig. 4). The reticulum cells were ill defined and relatively inconspicuous. There were still other nodes that showed proliferation of reticulum cells to the exclusion of other cells and these varied considerably in shape and size (Fig. 5). Some were branched and their processes blended with the underlying reticulum fibrils, but most were detached and were irregularly shaped, rounded, or hexagonal. The cytoplasm of these cells was pink and abundant, and their nuclei were small or large, evenly stained or hyperchromatic, and occasionally they were in a state of mitosis. The only other cells present were a few scattered mature lymphocytes and occasional polymorphonuclear leukocytes. The portal canals showed large infiltrations of cells of the variety typical of Hodgkin's disease. Sections of the bone marrow showed similar cells but in addition there were large areas of necrosis and a diffuse early fibrosis. The spleen showed considerable hemosiderosis and some fibrosis but no other specific changes.

Summary. Thus the changes in the lymph nodes of this patient were those of lymphosarcoma in May, 1941, while they were those of Hodgkin's disease in September, 1943. Autopsy in December, 1943, showed lymphosarcoma, Hodgkin's disease and reticulum cell sarcoma.

Case 2

In May, 1940, a white woman, 38 years old, was admitted complaining of cervical lymphadenopathy and hematuria of 2 months' duration. A roentgenogram of the chest disclosed a mass in the anterior mediastinum. In spite of x-ray therapy she gradually developed increasing pallor, a persistent cough and loss of weight, and died in December, 1942.

A well encapsulated cervical lymph node measuring 1.5 cm. in diameter, removed on May 9, 1940, was composed of homogeneous gray

tissue with no gross areas of hemorrhage or necrosis. Microscopically, a portion of the node showed a diffuse infiltration with eosinophils; fewer lymphocytes, plasma cells and polymorphonuclear leukocytes; and clusters of typical Sternberg-Reed cells (Fig. 6). Faintly stained, drawn-out reticulum cells were sparse and fibrosis was slight to moderate. The remainder of the node was composed of circumscribed "tubercle-like" areas which consisted from the center outward of a small focus of eosinophils and polymorphonuclear leukocytes, a zone of necrosis, a rim of loosely arranged Sternberg-Reed cells, and finally a band of dense fibrous tissue.

On December 30, 1941, a mass of gray neoplastic tissue measuring 2.5 by 1 cm. was removed from the orbit. Histologically, practically all of the cells were of the large lymphocytic variety (Fig. 7). Mitotic figures were quite abundant. Occasionally, ill defined, poorly stained reticulum cells and a few thin-walled capillaries were seen in the background. There were rare polymorphonuclear leukocytes but no Sternberg-Reed cells were seen.

Necropsy performed on December 4, 1942, showed emaciation and protrusion of the left eye. The lymph nodes of the left axilla, the mediastinum and the abdomen were variably enlarged, reaching their maximum size in the epigastrium where they measured 14 by 12 by 8 cm. In the region of the thymus they formed a flat mass, 10 by 6 by 1.5 cm. Everywhere they were matted together, firm and uniformly gray. None showed necrosis or hemorrhage. The spleen weighed 250 gm. and contained circumscribed gray tumor nodules measuring as much as 1 cm. in diameter. Similar but larger tumor masses were present in the left lung. Some of these, however, showed central areas of necrosis. The remaining organs, including the liver and vertebrae, showed no pertinent abnormalities.

Microscopical sections of many of the nodes showed a pathological process similar to that found in the orbital mass described above (Fig. 8). Nearly all of the cells were of the large lymphocytic series. There were only a few scattered polymorphonuclear leukocytes and plasma cells and no Sternberg-Reed cells. In other areas, however, and in the lungs the cells consisted of various proportions of lymphocytes, eosinophils, polymorphonuclear leukocytes, plasma cells and occasional Sternberg-Reed cells. There were also foci of necrosis. Sections of the nodules in the spleen were similar to those from the cervical lymph node described above except that there were more areas of necrosis and fibrosis. Some sections of the lumbar vertebrae showed areas composed entirely of lymphocytes while others contained, in addition,

eosinophils and plasma cells. There was an extensive purulent bronchopneumonia.

Summary. Biopsy in May, 1940, showed Hodgkin's disease of a granulomatous type. Biopsy in December, 1941, disclosed a picture resembling lymphosarcoma. Autopsy in December, 1942, showed a combination of the two processes.

Case 3

A white boy, 17 years of age, was admitted in March, 1942, complaining of a mass in the neck. This had first appeared 8 years previously following an attack of measles but had grown rapidly 1 month prior to admission. In spite of x-ray therapy he gradually developed weakness, pallor, loss of weight, an enlarged liver, pains in the back and legs and anemia, and died in September, 1943. Repeated sternal punctures showed an increasing replacement of the bone marrow with lymphoid tissue.

A group of "enlarged," loosely bound, discrete cervical nodes was removed on April 10, 1942. These nodes were uniformly pinkish gray and showed no necrosis or hemorrhage. Microscopically, the normal architecture was completely replaced by a diffuse and dense infiltration of mature lymphocytes (Fig. 9). Throughout the sections there were occasional thick-walled and thin-walled capillaries, surrounding which there was some increase in collagen. Attached to the latter were scattered, faintly stained, ill defined reticulum cells. There were no other cells present.

Necropsy was performed on September 27, 1943. The lymph nodes of the submaxillary regions, the supraclavicular fossae and the left infraclavicular fossa were enlarged to 1.5 cm. in diameter. The mediastinal and all abdominal nodes were much enlarged and matted together, reaching their greatest size along the abdominal aorta where they measured 16 by 6 by 4 cm. On section they were uniformly gray and showed no areas of necrosis or hemorrhage. The spleen weighed 810 gm.; the liver, 1790 gm.; and the kidneys, 120 gm. each. Each of these organs contained gray nodules of varying sizes.

Microscopical sections showed a complete disappearance of the normal architecture. There was some increase of reticulum cells. Many of these were elongated and their borders were either branched or otherwise irregular; others were somewhat rounded. All contained an abundant amount of cytoplasm and had round, regularly stained nuclei. Lymphocytes were greatly reduced in numbers but both the large and small variety were present. The remaining cells consisted of polymorphonuclear leukocytes, plasma cells and many Sternberg-Reed cells containing from 1 to 20 piled-up nuclei (Fig. 10). The outlines of

these giant cells were usually round but occasionally their borders were branched, assuming the appearance of reticulum cells. In addition to cells of the above variety, there were scattered areas of early necrosis. Sections of the spleen, liver and bone marrow showed essentially the same microscopical appearance.

Summary. Biopsy of a cervical lymph node showed a lymphosarcoma of the small cell variety. Autopsy 1½ years later disclosed typical Hodgkin's disease.

Case 4

A white man, 57 years old, was admitted to the hospital in September, 1943, complaining of general lymphadenopathy, cough, weakness, dyspnea, substernal pain and a mass in the abdomen, all of 3 months' duration. There was slight anemia and a differential count showed a few myelocytes and normoblasts. Pleural fluid contained "lymphosarcoma" cells. He died on October 23, 1943.

A group of lymph nodes removed from the groin on September 14, 1943, measured 3 cm. in diameter. They were well encapsulated, moderately firm and pinkish gray. Microscopically, the normal architecture was completely replaced by a diffuse infiltration of large, and in fewer numbers, small, lymphocytes (Fig. 11). The former showed mitosis and occasionally some fragmentation. Thick-walled capillaries with an increase of surrounding collagen were inconspicuous. Scattered sparsely throughout the nodes were a few faintly stained, ill defined reticulum cells. There was no other pleomorphism.

Autopsy performed on October 23, 1943, showed considerable emaciation. The axillary, clavicular and inguinal lymph nodes measured as much as 5 cm. in diameter, while those of the mediastinum and abdomen were about half that size. They were all moderately firm, pinkish gray and showed no necrosis or hemorrhage. The spleen weighed 290 gm. and the liver, 1390 gm. The latter contained several nodules of light pinkish gray tissue which measured as much as 5 cm. across. There were similar but smaller nodules in each lower lobe of the lungs. In addition, the left pleura was studded with many small, flat tumor masses and the corresponding pleural cavity contained 1000 cc. of blood-tinged fluid. The kidneys contained ill defined, light gray foci measuring less than 5 mm. across. The remaining organs were essentially normal.

Microscopical sections of most of the lymph nodes showed a complete replacement of the normal architecture with a mixture of large and small lymphocytes, plasma cells, polymorphonuclear leukocytes, a few eosinophils and many Sternberg-Reed cells (Fig. 12). There were, in addition, large phagocytes containing nuclear fragments. In some nodes nearly all of the cells were large lymphocytes, scattered amongst

which were very occasional phagocytes and Sternberg-Reed cells. The reticulum in these nodes was increased. Sections from the liver, kidneys, lungs and pleura showed lesions typical of Hodgkin's disease.

Summary. Sections of the peripheral nodes obtained for biopsy showed lymphosarcoma, whereas sections of most of the internal nodes examined at autopsy 1½ months later disclosed Hodgkin's disease. A few, however, still possessed some features of a lymphosarcoma.

Case 5

A white woman, 65 years of age, was admitted to the hospital in August, 1943, complaining of weakness, generalized lymphadenopathy and loss of weight for 2 years, and of an enlarged, tender spleen for 2 months. A severe progressive anemia preceded death in December, 1943.

Several matted axillary lymph nodes, measuring 3 cm. in diameter, were removed on September 9, 1943. They were well encapsulated and uniformly pinkish brown. Microscopical sections disclosed a complete replacement of the normal architecture with mostly large, but also a few small, lymphocytes (Fig. 13). Scattered throughout the parenchyma were varying numbers of reticulum cells which were either indistinct and united with the fibrils, or were separated off and either round or polygonal. Their cytoplasm was pink and abundant and their nuclei were evenly stained. They did not resemble Sternberg-Reed cells. There was no other pleomorphism.

Autopsy performed on December 24, 1943, disclosed considerable emaciation and jaundice. The lymph nodes in the mediastinum and throughout the abdomen were enlarged to 3 cm. in diameter, but showed little matting. They were firm and, except for central areas of necrosis in the thoracic nodes, uniformly pinkish gray. The spleen weighed 1490 gm. It was firm, dull reddish gray and contained numerous grayish nodules measuring as much as 3 mm. in diameter. The marrow of the lower thoracic and lumbar vertebrae was dull red and disclosed many irregular gray foci measuring up to 0.5 cm. in diameter. The left pleural cavity contained 1500 cc. of clear straw-colored fluid. The rest of the organs, including the liver and kidneys, were grossly normal.

Microscopically, the lymph nodes showed typical lesions of Hodgkin's disease (Fig. 14). Their normal architecture was completely replaced with varying numbers of large lymphocytes, plasma cells, polymorphonuclear leukocytes, occasional eosinophils and many Sternberg-Reed giant cells. Phagocytes containing nuclear fragments and hemosiderin were quite prominent. There were areas of necrosis and slight to moderate fibrosis. The spleen and bone marrow were diffusely involved with a similar process. There was an infiltration of lymphocytes.

plasma cells, polymorphonuclear leukocytes and occasional eosinophils between the liver cords. The latter were either partially or completely destroyed. The portal areas contained an increase of fibrous tissue and lymphocytes.

Summary. Biopsy of an axillary lymph node disclosed a lymphosarcoma with some added features of a reticulum cell sarcoma. Examination of the internal lymph nodes at autopsy 3½ months later showed Hodgkin's disease.

Case 6

A white man, 53 years old, was admitted to the hospital in December, 1942, complaining of weakness and lymphadenopathy of recent origin. He had enlargement of the spleen and liver. X-ray therapy was followed by some improvement until March, 1943, when weakness, anorexia, loss of weight and a diffuse inflammation of the skin of the lower abdomen and upper thighs became incapacitating. He died on May 5, 1943.

A group of nodes from the left inguinal region removed on December 12, 1942, measured 4 by 2 cm. The nodes were matted together and moderately firm. Cut surfaces showed pinkish gray tissue streaked with very recent hemorrhage. Microscopically, there was a complete, thin, peripheral rim of densely packed, large and small lymphocytes. The former showed many mitoses. Occasionally a faintly stained reticulum cell was present in the background. In the central portions of the nodes the cells were less numerous (Fig. 15). They were larger than large lymphocytes but smaller than the typical reticulum cells seen in case 1. Their nuclei were usually evenly stained although some were in a state of mitosis. Their cytoplasm was pink and prominent. No other cell types were present.

Autopsy on May 5, 1943, showed enlargement of the cervical, axillary, inguinal, mediastinal and abdominal lymph nodes to as much as 3 cm. in diameter. They were matted together and firm. Their cut surfaces were uniformly pinkish gray and showed no areas of hemorrhage or necrosis. The liver weighed 2740 gm. The portal areas were prominent and contained bands of gray tissue measuring 1 to 2 mm. across. The left pleural cavity contained 1700 cc. of straw-colored fluid. The rest of the organs, including the spleen and bone marrow, were grossly normal.

Microscopically, some of the nodes were identical with those removed on December 12, 1942, and described above, with the exception that the peripheral rim of cells was absent and all of the cells were similar to those seen in the central portions of those nodes. There were other lymph nodes, however, which showed considerable pleomorphism (Fig. 16). In addition to the large cells described above there were polymorphonuclear leukocytes, plasma cells, occasional eosinophils

and many Sternberg-Reed giant cells. Infiltrations of similar cells were found in the spleen, the portal canals of the liver, the cortices of the kidneys, the skin, and in and about the bronchi.

Summary. Biopsy in December, 1942, showed a transition between a large cell lymphosarcoma and reticulum cell sarcoma. Autopsy 6 months later disclosed, in addition, typical Hodgkin's disease.

Discussion

When a primary tumor in a lymph node is a typical lymphosarcoma, Hodgkin's disease, or reticulum cell sarcoma, there is no difficulty in making a definite diagnosis. This is not so, however, when the cellular composition is not of the type ordinarily designated as characteristic of one of the three diseases and often one encounters such combinations of cells that it is virtually impossible to label the tumor definitely. Furthermore, these combinations can be explained only by considering the three diseases as not only closely related but as having a common neoplastic origin, differentiating along one line in one lymph node or a portion of that node and along another line in another node or a portion of the same node. Or, it may be that for a time differentiation proceeds in one direction in all of the nodes and then as a result of treatment or spontaneously it proceeds in an entirely different direction.

The above considerations are well supported by the cases presented. Thus in case 1 a diagnosis of Hodgkin's disease was first made and 1 year later a diagnosis of lymphosarcoma was made on another lymph node. Two and one-half years later a third node again showed typical Hodgkin's disease while autopsy 1⅓ years later showed the changes of lymphosarcoma in some nodes, of Hodgkin's disease in others and of reticulum cell sarcoma in still others. It might well be argued that, because of the presence in the latter nodes of a few lymphocytes and occasional polymorphonuclear leukocytes, the process is a sarcomatous type of Hodgkin's disease. Yet if such a node were seen as a biopsy specimen one would not be justified in making any other diagnosis than that of reticulum cell sarcoma.

In case 2 the lesion seen on May 9, 1940, was in all respects the typical granulomatous form of Hodgkin's disease, abounding not only in eosinophils but also in Sternberg-Reed cells. A year and one-half later another biopsy showed a diffuse infiltration with regular large lymphocytes exhibiting much mitotic activity and only occasional polymorphonuclear leukocytes and a few underlying reticulum cells. Without the presence of Sternberg-Reed cells, which, according to Potter,[16] is the one specific feature of Hodgkin's disease, a diagnosis other than lymphosarcoma could not have been made. Certainly such a diagnosis

cannot be excluded because of the presence of regular reticulum cells in the background for they are present in normal lymph nodes. The same combination of large and small lymphocytes with a few reticulum cells scattered throughout the sections and the absence of eosinophils and Sternberg-Reed cells in cases 3, 4 and 5 accounted for the diagnosis of lymphosarcoma in the original specimens taken for biopsy. These cases might just as easily have continued to show the same picture or have been transformed into an outright reticulum cell sarcoma instead of developing into Hodgkin's disease. The alternative would be to call them atypical Hodgkin's disease which to us seems to be merely playing with words and evading the fundamental issue.

Finally, case 6 illustrates a neoplasm with cells larger than a large lymphocyte and smaller than an ordinary reticulum cell. The first biopsy showed, in addition, a peripheral rim of neoplastic tissue composed of typical large and small lymphocytes. Into what category can such a combination be placed? The lesion is neither an outright lymphosarcoma nor is it an outright reticulum cell sarcoma. It is half way between. Yet at autopsy 6 months later, while most of the nodes were composed of the large cells previously referred to, there were some which showed considerable cellular pleomorphism together with typical Sternberg-Reed cells. In these the changes were surely those of Hodgkin's disease. Thus, as already stated, it appears that the only logical explanation is to regard lymphosarcoma, Hodgkin's disease and reticulum cell sarcoma as ultimately arising from a common stem cell—the reticulum cell—and then according to further differentiation or lack of differentiation forming either combinations of these diseases or distinctly outright pictures of one type or the other.

If we accept the fact that these variations in cytological morphology are traceable to changes in differentiation of one cell type—the reticulum cell—then we must consider the presence of at least two stimulators that can bring about such changes in differentiation. These tumors are highly complex in cellular composition; therefore, if they are related to lymphosarcoma, part of the cellular proliferation that occurs in them must be brought about by the same factor that accounts for proliferation in the lymphosarcomas. Proliferation arising because of this stimulator would be lymphoid. The presence of myeloid cells such as neutrophils, eosinophils and possibly Sternberg-Reed cells is an indication of a second stimulator, one that would bring about myelopoiesis.

Thus, the variability in cellular components in these tumors may be accounted for by the local and/or general variations in activity of the myeloid and lymphoid stimulator substances. It has been shown that there are two such substances, one of which is specific for stimulating

the proliferation of myeloid cells, the other is specific for stimulating the proliferation of lymphoid cells.[15] These substances appear to be mutually reciprocal in action, *i.e.*, the myeloid stimulator causes the maturation of lymphoid cells and the lymphoid stimulator causes the maturation of myeloid cells. These substances have been found to occur in increased amounts in nearly equal proportions in the urine of patients with Hodgkin's disease and monocytic leukemia. Both are present, also, in the lipids of normal beef liver.[17] Crude extracts of the urine of patients with Hodgkin's disease and crude extracts of the lipids of normal beef liver produced pleomorphic lesions in the organs of guinea-pigs when such material was injected into these animals. Chemical separation of these extracts into carbinols and noncarbinols resulted in two equally potent fractions. The carbinols in each instance elicited a response of increased lymphopoiesis and the noncarbinols that of increased myelopoiesis. Noncarbinols and carbinols obtained from the urine of patients with chronic leukemia were given simultaneously to guinea-pigs and the response was similar to that obtained when crude unseparated extracts were given. The lesions elicited were not those typical of Hodgkin's disease, but in the livers, lungs, adrenals, kidneys and spleens of these animals there was hyperplasia of the lymphoid elements and a stimulation of the reticulum cells. The reticulum cells occasionally had more than one nucleus. Frequently there were a large number of eosinophils among the lymphoid cells and a few neutrophils. The lesions in the liver frequently were around blood vessels. An increased number of fibroblasts was seen in many of the lesions.

Extracts of the urine of seven patients with Hodgkin's disease were used in this work. Each gave about the same biological response with one exception, namely, case 1 of this report. Urine from this patient, obtained and extracted at the time that the diagnosis of lymphosarcoma was made on biopsy material, gave a response in the guinea-pig of increased lymphopoiesis only.

This work and the changing picture presented by these cases lead us to question how we can best describe Hodgkin's disease. Healing and regression that involve the proliferation, maturation and destruction of many of the cellular elements of the blood occur in Hodgkin's disease, and this process continually repeats itself as the neoplasm grows. A system of three parts is necessary for the activation of this process: (1) the presence of mesenchymal stem cells; (2) an excess of the two substances capable of stimulating proliferation of the stem cells so that myeloid and lymphoid proliferation occur simultaneously; and, (3) such substances should not be in such excess as to preclude

their reciprocal action, but would allow for a certain amount of maturation to take place. This system allows typical Hodgkin's disease to develop. It is possible that locally there might be an excess of one substance and therefore a few nodes would be typical of lymphosarcoma or the bone marrow would be hyperactive in myelopoiesis. A general excess of one substance might occur for a short time and later be followed by an excess of both. Either of these explanations might account for the variations encountered in these cases. If the excess of both substances were so great locally that stimulation of cellular proliferation without maturation occurred, then the result would be reticulum cell sarcoma. If equally great excesses were evidenced generally, then monocytic leukemia would be the result.

Other factors are probably involved, but for the most part this system seems to explain not only typical Hodgkin's disease, but also the variations of the disease that are reported here.

Summary

Six cases are presented that at one time during the lives of the patients were diagnosed as Hodgkin's disease and at another time as lymphosarcoma and that at autopsy showed various combinations of Hodgkin's disease, lymphosarcoma and reticulum cell sarcoma. These various combinations can be explained only by considering the three diseases as arising from a common stem cell—the reticulum cell—and then differentiating in one direction or another according to the amount and type of stimulation. Proliferation arising because of an excess of the lymphoid stimulator gives rise to a lymphosarcoma while that due to an excess of both lymphoid and myeloid stimulators gives rise to Hodgkin's disease, providing the stimulation is accompanied by maturation, or to reticulum cell sarcoma when unaccompanied by maturation. Combinations of these diseases result when the specific stimulators are not uniformly distributed throughout the organs or when a temporary excess of one is followed by a temporary excess of the other.

We are indebted to Dr. C. J. Bucher for allowing us to use the surgical material and to Dr. Joseph Stasney who performed the autopsy in case 3.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

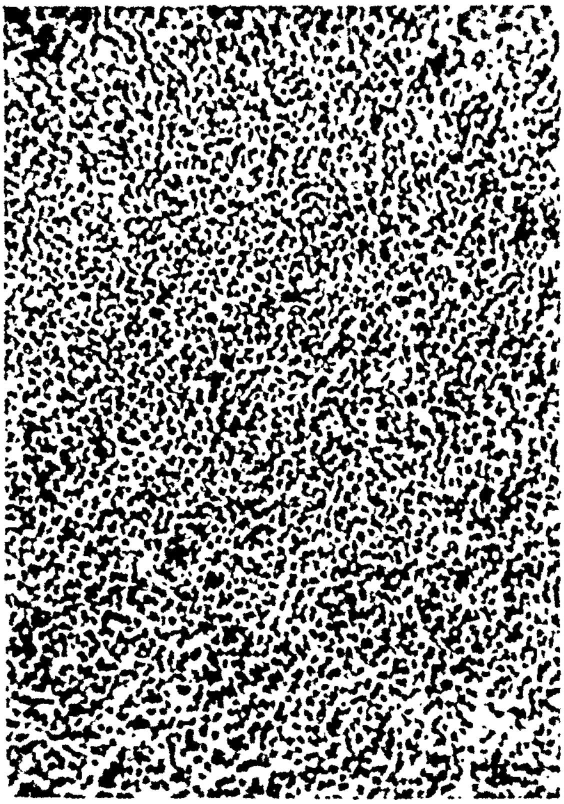
PLATE 37

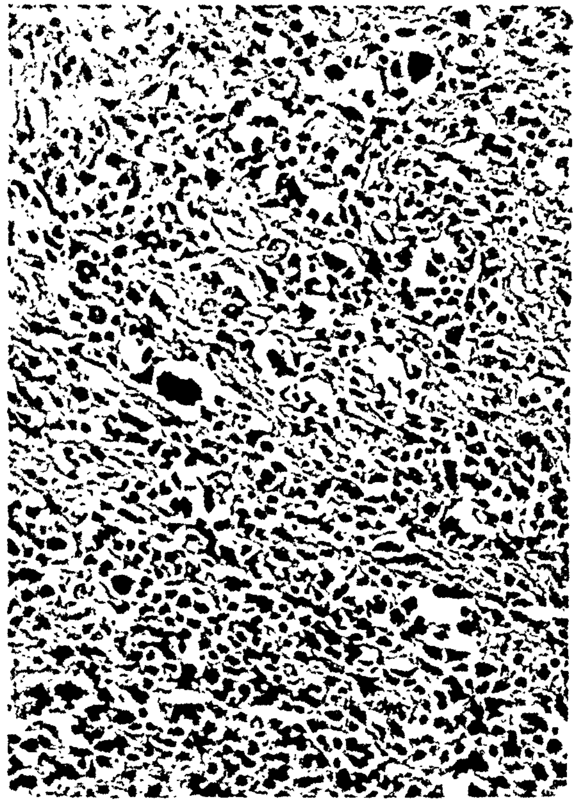
FIG. 1. Case 1. Section of a lymph node removed in May, 1941, showing a diffuse infiltration with lymphocytes. Hematoxylin and eosin stain. × 200.

FIG. 2. Case 1. Section of a lymph node removed in September, 1943, showing moderate fibrosis, and a diffuse infiltration with lymphocytes, plasma cells, a few polymorphonuclear leukocytes, scattered eosinophils and typical Sternberg-Reed giant cells. Hematoxylin and eosin stain. × 200.

FIG. 3. Case 1. Section of a lymph node obtained at autopsy in December, 1943, showing a slight increase in fibrous tissue and a diffuse infiltration with mature lymphocytes. Hematoxylin and eosin stain. × 200.

FIG. 4. Case 1. Section of a lymph node obtained at autopsy in December, 1943, showing considerable fibrosis and an infiltration with lymphocytes, plasma cells, polymorphonuclear leukocytes, occasional eosinophils and Sternberg-Reed giant cells. Hematoxylin and eosin stain. × 200.





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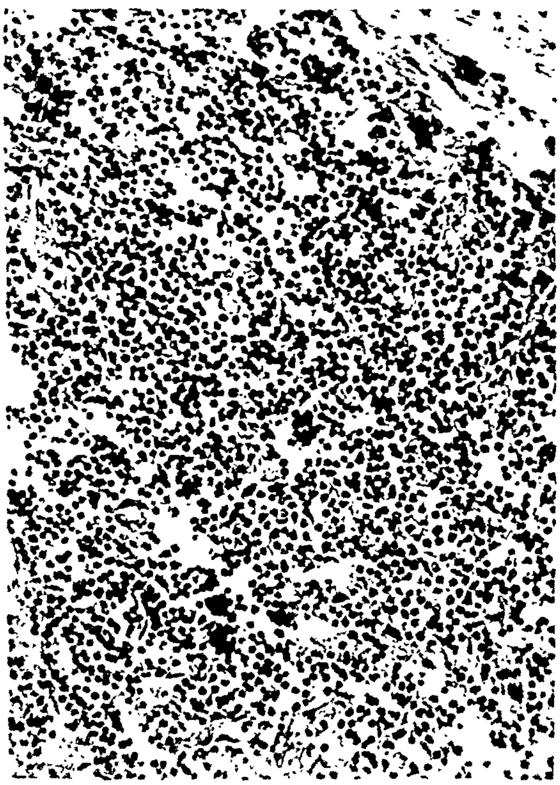

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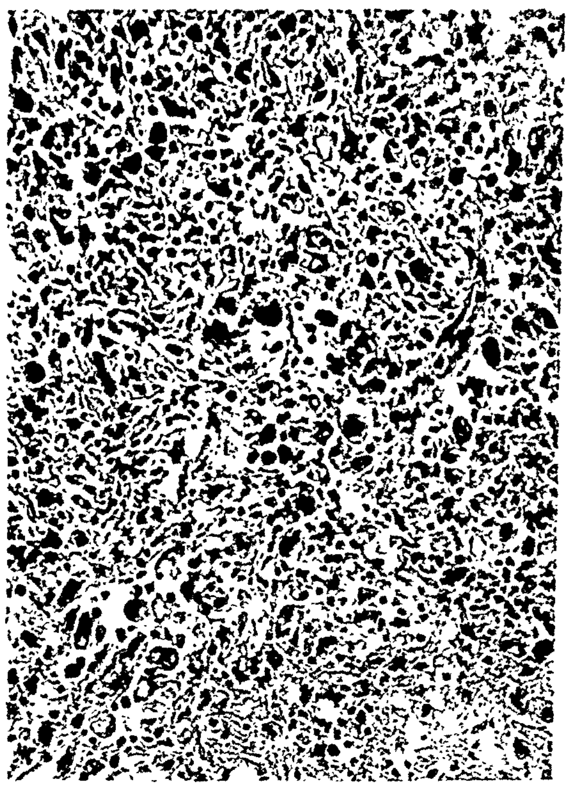

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Herbut, Miller and Erf Relation of Hodgkin's Disease

PLATE 38

FIG. 5. Case 1. Section of a lymph node obtained at autopsy in December, 1943, showing an almost pure proliferation of reticulum cells. There are also present a few scattered lymphocytes. Hematoxylin and eosin stain. × 200.

FIG. 6. Case 2. Section of a lymph node removed in May, 1940, showing a diffuse replacement of the normal architecture with eosinophils; fewer lymphocytes, plasma cells and polymorphonuclear leukocytes, and clusters of typical Sternberg-Reed cells. Hematoxylin and eosin stain. × 200.

FIG. 7. Case 2. Section of a lymph node removed in December, 1941, showing mostly large lymphocytes; scattered small lymphocytes and a very occasional, poorly stained reticulum cell in the background. Hematoxylin and eosin stain. × 200.

FIG. 8. Case 2. Section of a lymph node obtained at autopsy in December, 1942, showing a diffuse infiltration with large lymphocytes, lesser numbers of small lymphocytes and only an occasional polymorphonuclear leukocyte. Hematoxylin and eosin stain. × 200.

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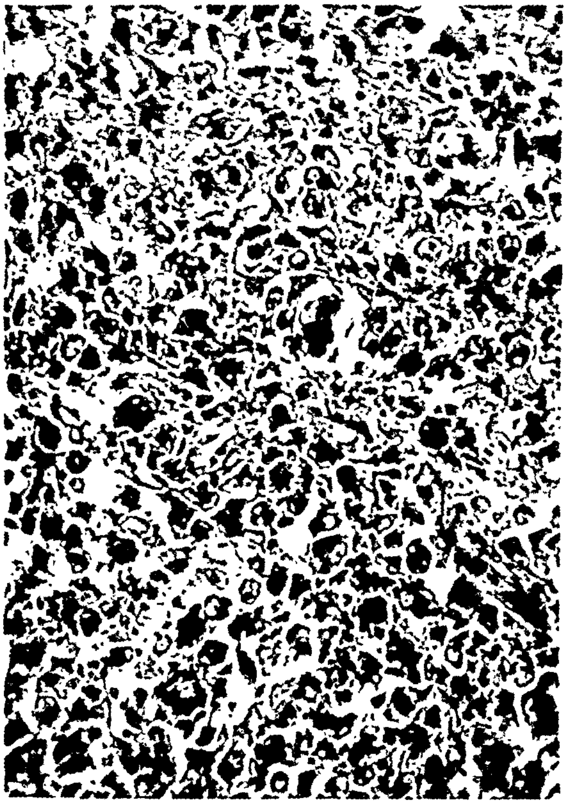

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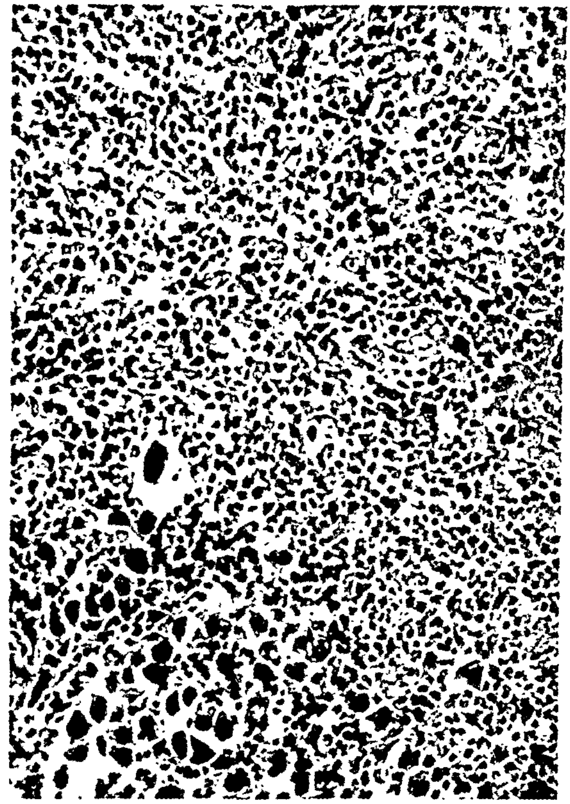

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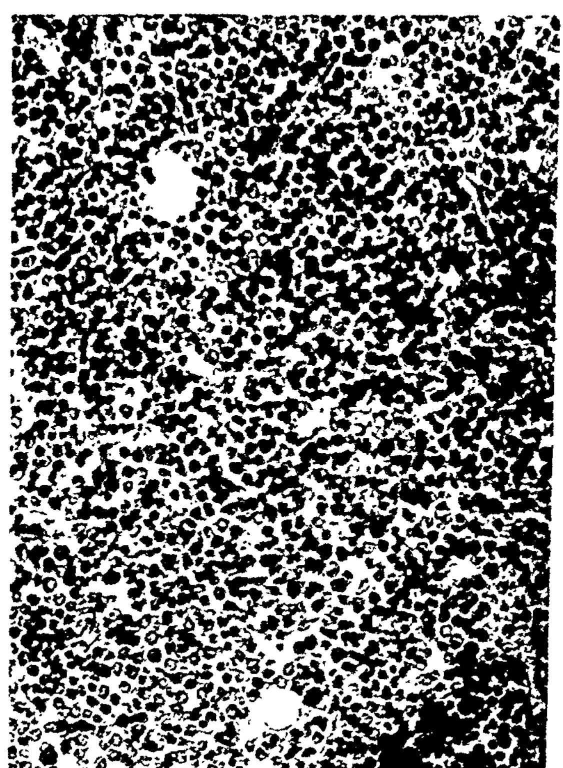

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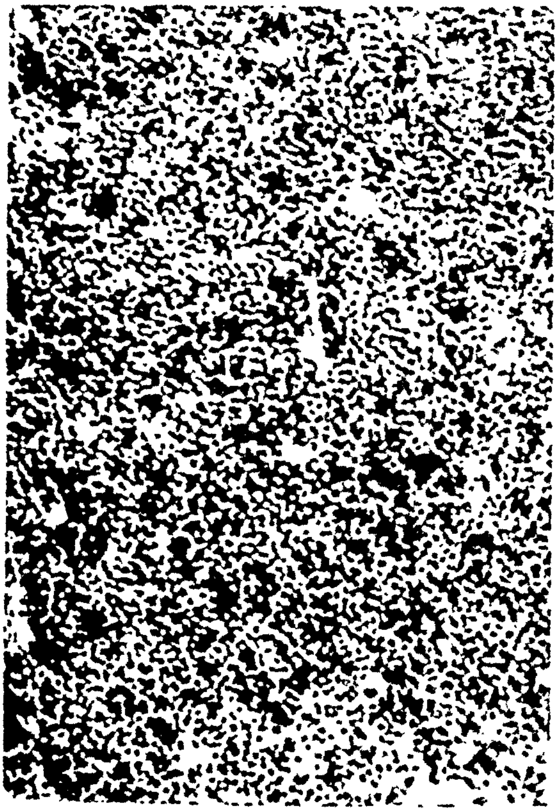
PLATE 39

FIG. 9. Case 3. Section of a lymph node removed in April, 1942, showing a dense infiltration with mature lymphocytes. In the background there are a few ill defined, faintly stained reticulum cells. Hematoxylin and eosin stain. × 200.

FIG. 10. Case 3. Section of a lymph node obtained at autopsy in September, 1943, showing a slight increase in reticulum and a diffuse infiltration with large and small lymphocytes, polymorphonuclear leukocytes, plasma cells and typical Sternberg-Reed giant cells. Hematoxylin and eosin stain. × 200.

FIG. 11. Case 4. Section of a lymph node removed in September, 1943, showing a diffuse infiltration with lightly and darkly stained large and small lymphocytes. Occasionally the nuclei of the former are fragmented. Hematoxylin and eosin stain. × 200.

FIG. 12. Case 4. Section of a lymph node obtained at autopsy in October, 1943, showing large and small lymphocytes, plasma cells, polymorphonuclear leukocytes, a few eosinophils and many Sternberg-Reed giant cells. There are also several large phagocytes laden with nuclear fragments. Hematoxylin and eosin stain. × 200.



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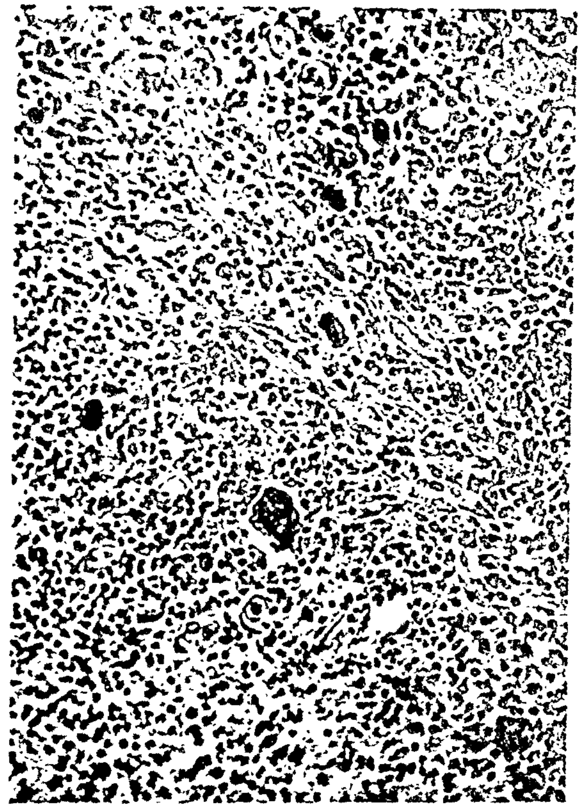

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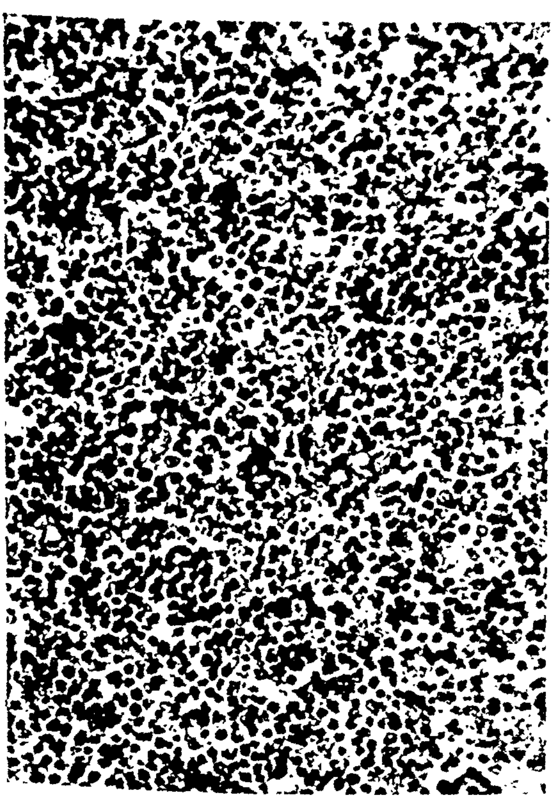

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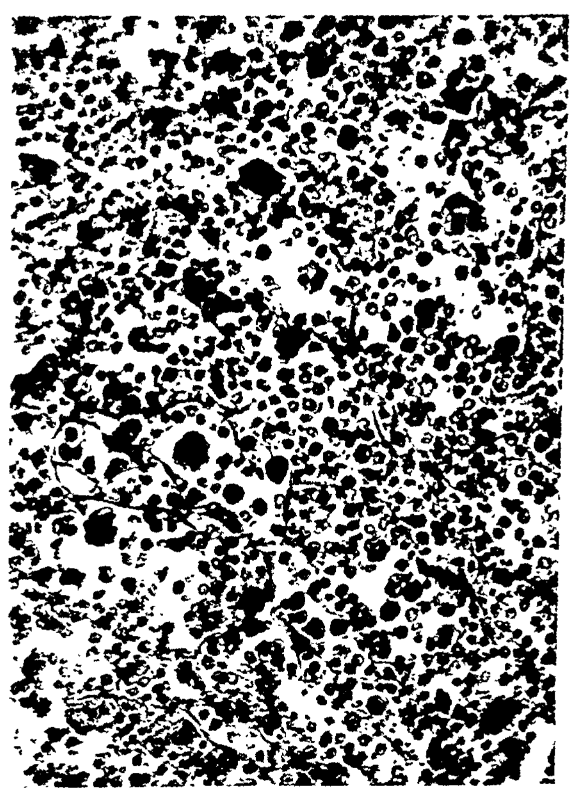

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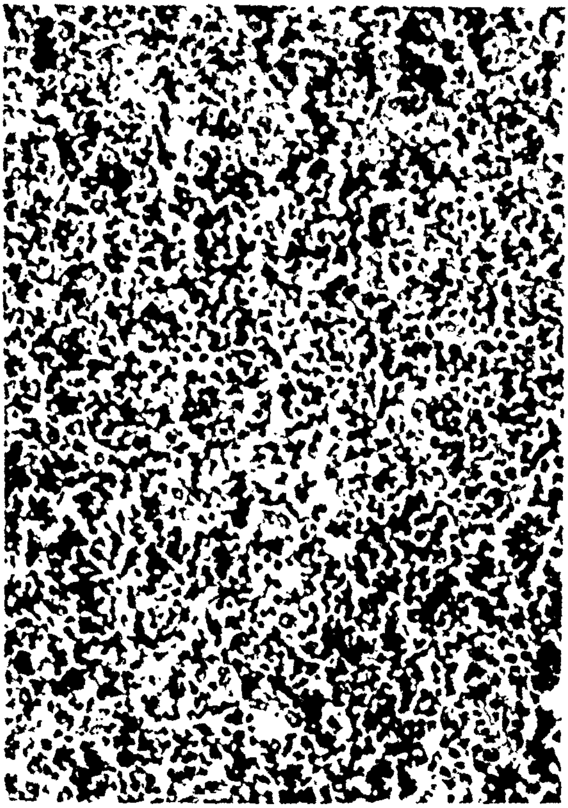
PLATE 40

FIG. 13. Case 5. Section of a lymph node removed in September, 1943, showing a diffuse infiltration with large and small lymphocytes. In the background there are scattered, faintly stained and ill defined reticulum cells. Hematoxylin and eosin stain. × 200.

FIG. 14. Case 5. Section of a lymph node obtained at autopsy in December, 1943, showing a diffuse infiltration with large lymphocytes, plasma cells, polymorphonuclear leukocytes, occasional eosinophils and many Sternberg-Reed giant cells. Hematoxylin and eosin stain. × 200.

FIG. 15. Case 6. Section of a lymph node removed in December, 1942, showing a diffuse infiltration with large cells which are larger than large lymphocytes but smaller than typical reticulum cells. Hematoxylin and eosin stain. × 200.

FIG. 16. Case 6. Section of a lymph node obtained at autopsy in May, 1943, showing large cells in Figure 15 and also polymorphonuclear leukocytes, plasma cells, occasional eosinophils and several Sternberg-Reed giant cells. Hematoxylin and eosin stain. × 200.



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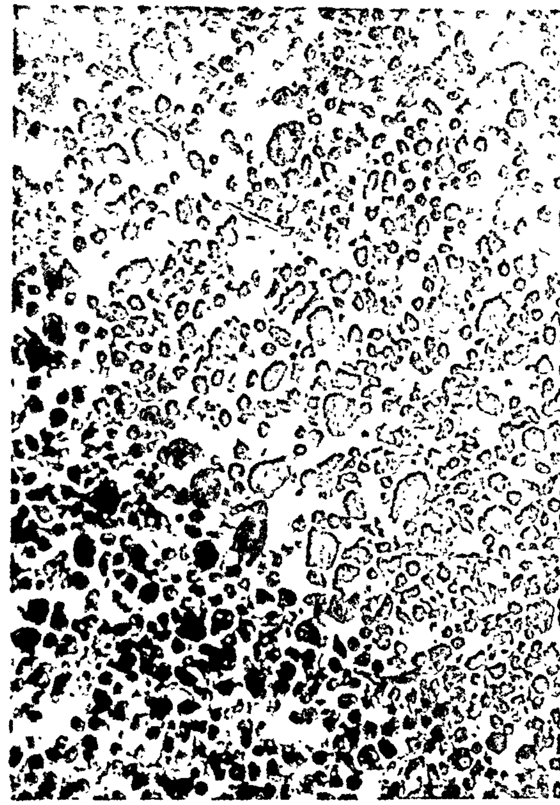

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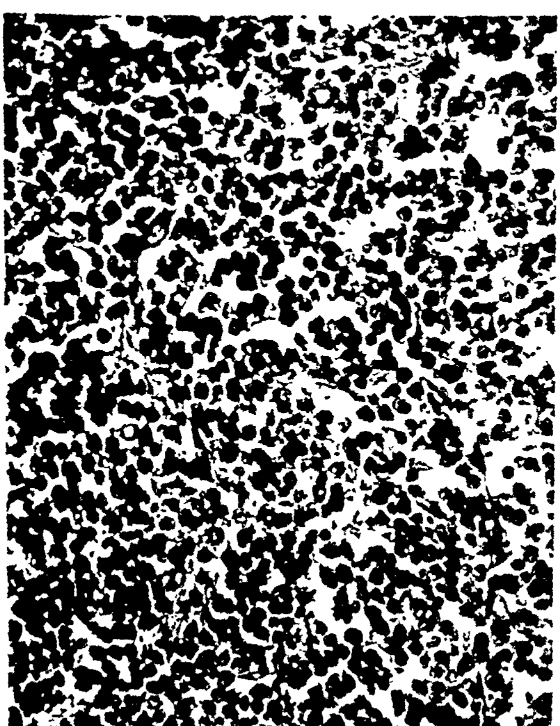

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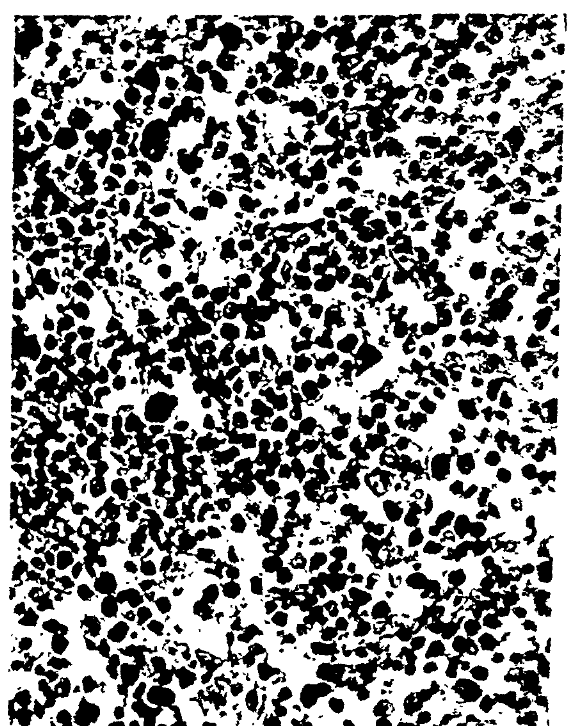

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ARREST AND REPAIR IN EXPERIMENTAL ENDOCARDITIS LENTA *

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In previous papers we have shown some of the changes observed very early[1] after rabbits had been inoculated intravenously with streptococci isolated from the blood of human patients with endocarditis and also some of the more advanced lesions[2] when the vegetations of the valve leaflets had attained large size. From these studies it has been possible to gain an idea of the successive stages in this infectious disease as it attacks the cardiac valves in the experimental animal and progresses to a fatal termination. It should be acknowledged, however, that only fixed pictures or stages of the process have been observed and that the relation of one stage to another has been surmised rather than actually seen, a defect which is inherent in purely morphological studies and not fully escaped even when such studies have been made upon experimental material, in which the individual experiment has been terminated at will.

When we undertook to examine the instances of apparent arrest and repair of the lesions in these experimental animals, even greater difficulties were encountered. That fibrous thickening and deformity, observed in an animal sacrificed several weeks or months after initiation of experimental inoculations, might be properly regarded as evidence that these alterations had resulted from the repair of a previously active lesion of the experimental disease is an assumption rather than an observed fact and should be accepted with reserve and even with suspicion until repeated observations of similar changes have supplied adequate confirmation. The probable nature of such an assumption is afforded some support by the recorded behavior of the animal and the laboratory tests made during the course of the disease, such as changes in temperature, in body weight, in the heart sounds and especially the results of cultures of the blood. The problem is much the same as that presented by the necropsy when a good clinical record of the case is available.

In general, experimental endocarditis, once established in the rabbit, tends to progress to a fatal termination. Nevertheless, one sometimes observes evidence of local arrest and local healing. Spontaneous re-

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covery in the rabbit appears to take place rarely if at all, perhaps about as often as it may occur in the human disease. By the application of therapeutic measures we have been able to alter the natural course of the infection. In many instances administration of large doses of serum, vaccines, sulfonamides and other chemicals was followed by the production of large valvular vegetations, extensive myocardial damage and early death. In recent years, however, greater therapeutic success has been attained in a few animals.

In the arrest of the disease and repair of the injured structures, various biological mechanisms are concerned. At the very onset, when the initial shower of streptococci has entered the circulating blood, there is evidence of a rapid phagocytosis of these bacteria by wandering cells and by the endothelial cells lining the blood vessels in general. The vast majority of the bacteria are killed and digested by the phagocytic cells without leaving any persistent anatomical alteration at the site of their destruction. Even after prolonged streptococcemia the liver ordinarily shows no recognizable structural alteration, although there can be no doubt that myriads of streptococci have been digested there. In the spleen, there is marked enlargement due to increase of all the elements, but especially to congestion, and after prolonged infection there is evidence of progressive fibrosis about the follicles. In the lungs there is early thickening of all the alveolar septa with formation of septic capillary thrombi and also a conspicuous endarteritis with edematous thickening, perivascular edema and hemorrhages along the branches of the pulmonary arteries. The lungs receive and destroy large numbers of the injected streptococci, which are abundantly held in the pulmonary capillaries. In the heart the bacteria are ordinarily fixed by the endocardium and in the myocardial capillaries to a lesser extent, but while the spleen, liver and lungs destroy the bacteria, usually without trace, the lining endothelium of the cardiac cavities and of the myocardial vessels often fails to exterminate completely the streptococci deposited here. It seems that the pressure of contact of the valve surfaces during the movement of the heart favors the persistence of the bacteria at the contact sites. In the myocardium, endothelial phagocytosis of the streptococci is associated with swelling of the endothelial cells concerned and this in turn diminishes the vitally essential supply of blood through the myocardial capillaries. Apparently on this account the bacteria tend to survive more readily in these situations.

Once there has been produced a mass of infected fibrin on the valve leaflet, the bacteria tend to grow in the fibrin as compact colonies and in these colonial masses they are evidently able to produce enough toxic substances to keep at a distance any phagocytic cells. As long as the

bacteria here are able to multiply rapidly in masses they defy all efforts of the host to destroy them.

It appears, however, that the proliferation of these streptococci may be influenced particularly by antagonistic substances present in the blood. Some of these antibacterial substances are produced by the tissues of the host. In untreated patients the blood culture may at one time develop a visible growth only after 3 or more days and in a later blood culture the growth may be distinct in less than 24 hours. The indicated bacteriostatic effect is even more evident in patients subjected to treatment with anti-infectious therapeutic agents. Evidently an antibacterial effect may be exerted upon colonies in the endocardial vegetations as well as upon cocci free in the circulating blood and their progeny in the culture flask. Is it possible that such agents in the circulating blood may influence the infected vegetations to such a degree that the bacteria no longer produce poisons in sufficient amounts to keep the healing elements at bay? We believe that this question may be answered in the affirmative. In some of the experimental animals, various stages of arrest and repair have been observed, sometimes in control animals but more often in treated animals.

The injury inflicted upon the streptococci is manifested by various degrees of bacteriolysis revealed by alterations in size, form and arrangement, by abnormal staining of the bacteria, and by their fragmentation and dissolution; also by the more intimate approach of phagocytic cells and the actual ingestion by them of the streptococci in the vegetation. Furthermore, the mass of infected fibrin no longer repels the advance of endothelial and fibroblastic cells, but now permits these healing elements to penetrate and surround it so as to wall in the crippled bacterial colonies and eventually produce a scar at the site of their extermination. Individual examples of arrest and repair may serve to illustrate the varied processes observed in the endocardium.

EXPERIMENTAL OBSERVATIONS

Rabbit 166, series 4B. Daily intravenous doses of living culture of Streptococcus faecalis (enterococcus), for 13 days; repeated positive blood cultures; abnormal heart sounds; treated with specific bacteriophage and thiobismol; death on the 30th day; large active mitral vegetation with local healing; healed aortic valvular lesion.

Rabbit 166, weighing 1665 gm., was inoculated with *Streptococcus viridans,* strain L 114-1, originally isolated from the blood of a patient who died of typical endocarditis lenta. The culture had been subsequently passaged in rabbits. The animal received intravenous injections of a heavy suspension of the living bacteria: 0.25 cc. on February

gressive vegetation on the mitral valve. A section through a swollen mitral leaflet is shown in Figure 8. At this site the bacteria have penetrated into the substance and have established a colony beneath the endothelium and this colony is surrounded by a loosely assembled wall of wandering cells. Figure 9 shows this colony and the adjacent structures in color. There are scattered streptococci in the substance of the valve about the bacterial colony and even at a considerable distance from it. Some cocci can be recognized within a polynuclear leukocyte about 50 μ from the endothelial surface at the right of the figure. Many of the cocci near the colony have been taken up by phagocytic cells and it would seem that the bacterial penetration may have come about through transport by wandering cells or possibly by movement of plasma in the intercellular spaces of the stroma after injury to the endothelial covering of the valve. A part of the bacterial colony at higher magnification is represented in Figure 10. Many of the streptococci at the periphery of the colony have failed to retain the Gram stain and some of the cocci within the phagocytic cells appear to be partly digested. In this untreated animal there is evidence of unsuccessful defense by the mechanisms of bacteriolysis, phagocytosis and partial encapsulation of the invading bacteria.

Rabbit 593, series 14A, was inoculated by intravenous injection of bacterial suspension, *Streptococcus viridans* (salivarius), strain CC, daily for 8 days, and was then allowed to remain without further inoculation and without treatment until death on the 18th day, when the body was immediately fixed by arterial perfusion. Various stages in healing of local lesions have been observed in untreated animals from time to time and these have been found most frequently in the right heart, on the mural endocardium, the tendinous cords, or the tricuspid leaflets. In rabbit 593, however, there was a well encapsulated, small, round vegetation situated near the free margin of the mitral leaflet (Fig. 11). This evidently developed in the usual way to produce a mass of infected fibrin attached to the auricular face of the leaflet and subsequently became encapsulated by growth and extension of endothelial and fibroblastic cells around and into the fibrin near the surface. These cells have also extended inward to approach the "unhappy" bacterial colonies and to ingest some of the bacteria. The periphery of the encapsulated vegetation is shown in Figure 12. Evidently the bacterial proliferation has been restrained by something in the animal's blood so that the living cells have been able to take possession of the superficial layers of the fibrin and to grow inward to come into intimate contact with the bacterial colonies. Again, however, the defense mechanisms have not been sufficiently powerful to exterminate the bacterial

invaders and, in fact, at other parts of the mitral valve of this animal more progressive lesions were still evident. In spite of a partial defense, the disease went on to a fatal termination.*

These few examples of the many animals in which we have observed partial arrest and partial healing of endocardial lesions may serve to introduce a consideration of some of the rabbits in which complete arrest and healing have occurred and may help in the undertaking and interpretation of the scars and deformities observed in these recovered animals.

Rabbit 392, series 5B, inoculated with an enterococcus strain of *Streptococcus viridans* and treated with specific bacteriophage and thiobismol, is an example of apparently complete arrest of the disease. A photograph of a gross section through the mitral orifice is shown in Figure 13. We regard the deformity of the mitral leaflets and the smooth, irregularly rounded nodules as sequelae of healed vegetations. Microscopical examination has failed to disclose any bacteria and the healing is evidently complete. Similar observations have been made in several rabbits, of which no. 133, series 2B, will be considered in more detail.

Rabbit 133, series 2B. Daily intravenous inoculations of living culture of Streptococcus faecalis for 6 days; repeated positive blood cultures for subsequent 12 days; later blood cultures, negative; treatment with bacteriophage and neoarsphenamine; in apparent health when sacrificed on 47th day; mitral valve healed with deformity.

Rabbit 133, series 2B, weighed 1500 gm. on December 23, 1942. It was inoculated with suspensions of bacterial culture, strain L 114-1, an enterococcus isolated from the blood of fatal human endocarditis and subsequently passaged through a rabbit. The inoculations were given intravenously, 2 cc. daily, December 25 to 30, inclusive, to a total amount of 12 cc., and then discontinued. The weight fell to 1355 gm. on December 30. Blood culture taken on December 31 was positive, as were subsequent blood cultures taken on January 6 and 11. Later blood cultures taken on January 19 and 25, and on February 3 and 9 remained sterile. The lowest weight observed was 1330 gm. on January 22; after that the weight increased again to 1560 gm. on February 10. Auscultation was negative on January 9 but on January 14 the first heart sound seemed defective and a diagnosis of mitral stenosis was

* Photomicrographs of this healing mitral lesion, as well as pictures of other healed and partially healed vegetations, were shown in the Scientific Exhibit at the meeting of The American Medical Association, Atlantic City, N. J., June 8 to 12, 1942. Program reference: Mac Neal, W. J., Spence, M. J., and Allen, A. C. Comparative study of the lesions of clinical and experimental endocarditis. Program of Scientific Assembly, p. 245, space 325; abstract in *J. A. M. A.*, 1942, 119, 71.

recorded. On January 15 electrocardiographic and stethographic records were made and these were repeated on January 19. Problematic departures from the normal graphs were observed. Subsequently, auscultation of the heart was recorded as negative on February 3 and again on February 10. At this time the animal appeared healthy and had obviously recovered from infection of the blood stream. It was sacrificed on February 10, on the 47th day after initial inoculation.

Because of the clinical evidence of recovery and also on account of the anatomical condition observed after death, the treatment of the animal is worthy of detailed presentation. Therapeutic measures were instituted on January 1, 1943, 2 days after inoculation with streptococci had been discontinued. A specific bacteriophage, prepared in our laboratory, was injected intravenously in a dose of 10 cc. on January 1 at 9:00 A.M. and again at 1:00 P.M.; on January 2 at 9:00 A.M., 4:15 P.M. and 8:30 P.M.; on January 3 at 8:10 A.M., 4:10 P.M. and 12:00 midnight. These injections were continued with variations in time intervals until February 9. In addition, neoarsphenamine was administered intravenously in several courses as shown in the tabulated protocol of treatment (Table I). In brief, specific bacteriophage was given intravenously two or three times a day up to February 1 and once a day thereafter; neoarsphenamine was given several times a day in four courses: January 2 to 7, January 11 to 13, January 17 to 19, and January 24 to 26. The doses were spaced throughout the entire 24 hours in the earlier period. We do not wish to emphasize the significance of this particular therapeutic program and the detail is set down as a matter of record. It was planned to resemble the schedule of Osgood.[3]

Rabbit 133 was exsanguinated, under ether anesthesia, by bleeding from the carotid artery, followed by perfusion with saline solution and finally Helly's formalized Zenker's solution for immediate fixation of the tissues in the distended state. This method of fixation removed most of the blood from the vascular system and prevented post-mortem lysis of tissue elements and bacteria. Culture of the carotid blood remained sterile. Gross examination at necropsy revealed numerous irregular thickenings and nodules along the free margins of the mitral leaflets and moderate thickening of the aortic and pulmonary valves; the tricuspid appeared normal. The liver contained a few lesions of coccidiosis. Lungs, spleen, kidneys and brain were negative. A gross photograph of this heart is not available. Its general appearance was essentially similar to that of several other hearts in this group of animals, one of which is shown in Figure 13 (rabbit 392, series 5B).

On microscopical examination the myocardium of rabbit 133 ap-

TABLE I

Rabbit 133, Series 2B; Therapy from January 1 to February 9, 1943

All medication was given by intravenous injection. Clock time is shown on the basis of 24 hours starting with zero at midnight.

Date	Bacteriophage, 10 cc. doses at clock time below					Neoarsphenamine Amount and clock time below
Jan. 1		9:00	13:00			
2		9:00	13:00	16:15	20:30	0.8 mg. at 9:00, 13:00, 16:15, 20:30
3		8:10		16:10	24:00	0.8 mg. at 8:10, 16:10, 24:00
4		8:10		16:10		0.8 mg. at 8:10, 16:10
5	0:10	8:30		16:30		1.8 mg. at 0:10, 8:30; 2.0 mg. at 16:30
6	0:50	8:05		16:10		2.0 mg. at 0:50, 8:05, 16:10
7	0:10	8:05		16:05		2.0 mg. at 0:10, 8:05, 16:05
8	0:05	8:25		14:25		2.0 mg. at 0:05
9		9:00		15:00		
10		11:00		15:00		
11		9:00	12:30	16:00	20:10	2.0 mg. at 9:00, 12:30, 16:00, 20:10
12		8:30		16:00	23:55	2.0 mg. at 8:30, 16:00, 23:55
13		8:30		16:10	23:55	2.0 mg. at 8:30, 16:10, 23:55
14		8:30		16:10		
15		9:00		15:30		
16		10:00		14:13		
17		8:30	12:00	16:00	19:45	1.8 mg. at 8:30, 12:00, 16:00, 19:45
18		8:30		16:00	24:00	1.8 mg. at 8:30, 16:00, 24:00
19		8:30		16:00	23:40	1.8 mg. at 8:30, 16:00, 23:40
20		9:00		16:00		
21		9:00		16:00		
22		9:00		17:00		
23		10:00		14:30		
24		8:00		16:40		1.8 mg. at 8:00, 16:30
25		9:30		16:30		1.8 mg. at 9:30, 16:30
26		9:30		16:30		1.8 mg. at 9:30, 16:30
27		9:30		17:30		
28		11:00		15:30		
29		9:00		19:00		
30		9:00		16:30		
31		10:00		16:00		
Feb. 1		10:00		18:00		
2		10:30				
3		10:30				
4				14:00		
5		11:00				
6		11:00				
7		9:00				
8		9:00				
9		10:00				

peared negative. The sections through the mitral leaflets are of special interest. The irregular thickening and deformity of the valves is shown in Figures 14, 15 and 17. Bacteria were not found. The nodules were made up of fibroblastic cells and fibrils, and were covered by endothelium. Well defined mitotic division figures were readily found in the fibroblasts and also in the endothelial cells. Wandering cells were present in the stroma in very small numbers, only a lymphocyte here and there, and there was a suggestion of slight edema in the leaflets. A section through the aortic valve is shown in Figure 16 and the prominent nodule on the leaflet is represented in the colored drawing, Figure 18. Here also there are mitoses in the fibroblasts. Evidently there was at the time of death an active reparative process in the aortic and mitral leaflets, which we interpret as healing of previously infected vegetations at these sites.

The sections of liver and of kidney showed no bacterial lesions. The spleen was well distended by the perfusion. Its pulp spaces were wide in the sections and the follicles relatively small. In the lungs there were scattered small foci of cellular infiltration about some smaller arterioles but in general the lungs were normal.

These observations on rabbit 133 may be open to various interpretations. We are convinced, however, that this animal did have bacterial vegetations on the mitral and aortic valves, evidenced by bacteremia persisting until January 11, 12 days after inoculations had been discontinued, and by abnormal heart sounds on January 14, 15 and 19, as well as by the anatomical deformity and active process of repair in the mitral and aortic valves after death of the animal. If this be accepted, then the negative blood cultures after January 11, taken on January 18 and 25, and on February 3 and 9, together with the healthy appearance of the animal and the progressive increase in weight after January 22, as well as the structural changes in the heart after the animal was killed on February 10, may be properly interpreted as proof of arrest of the disease and healing of the local lesions in the heart.

Rabbit 362, series 8B, inoculated with Streptococcus viridans (salivarius), strain F 330-7, daily for 23 days; treated with penicillin and thiobismol; death after 23 days; healed mitral endocarditis with newly formed capillaries and hematogenous pigment in the substance of the valve.

Rabbit 362, series 8B, was inoculated with *Streptococcus salivarius*, strain F 330-7, isolated from the blood of a patient with bacterial endocarditis and passaged in a rabbit with production of cardiac vegetations. The intravenous inoculations were started on August 12 and continued daily through September 3 in doses of 0.125 to 0.375 cc. of

bacterial suspension. The rabbit weighed 2300 gm. on July 29, and 2165 gm. on August 10. Cultures of the blood taken on August 18 and 25 and on September 1 were positive. The one taken on September 3 remained negative, in spite of intravenous injection of bacteria on the previous day. Auscultation of the heart, daily except for six scattered omissions, was negative throughout. On September 2 severe diarrhea

TABLE II

Rabbit 362, Series 8B; Therapy from August 12 to September 2, 1943

Penicillin was administered by intramuscular, and thiobismol by intravenous, injection. Clock time is shown on the basis of 24 hours starting with zero at midnight.

	Penicillin, doses of 20 units each on the hour at clock time below								Thiobismol, doses of 2 mg. each on the hour at clock time below	
Aug. 12				10	13	16	19	22	10	16
13	1	4	7	10	13	16	19	22	10	16
14	1	4	7	10	13	–	–	22	10	–
15	1	4	7	10	13	–	–	–	–	–
16	–	–	–	10	13	16	19	22	10	16
17	1	4	7	10	13	16	19	22	10	16
18	1	4	7	10	13	16	19	22	10	16
19	1	4	7	10	13	16	19	22	10	16
20	1	4	7	10	13	16	19	22	10	16
21	1	4	7	10	13	–	–	22	10	–
22	1	4	7	–	13	–	–	–	–	–
23	–	–	–	10	13	16	19	22	10	16
24	1	4	7	10	13	16	19	22	10	16
25	1	4	7	10	13	16	19	22	10	16
26	1	4	7	10	13	16	19	22	10	16
27	1	4	7	10	13	16	–	22	10	16
28	1	4	7	10	13	–	–	22	10	–
29	1	4	7	–	13	16	–	–	–	–
30	–	–	–	10	13	16	19	22	10	16
31	1	4	7	10	13	16	19	22	10	16
Sept. 1	1	4	7	10	13	16	19	22	10	16
2	1	4	7	10	13	16	19	22	10	16
3	1	Total 2820 units in 22 days							Total 70 mg. in 22 days	

appeared and the rabbit died at 1:37 A.M. on September 3. The body was immediately fixed by arterial perfusion.

Treatment included penicillin in intramuscular doses of approximately 20 Oxford units at intervals of 3 hours, together with thiobismol in intravenous doses of 2 mg. given twice daily, with intermissions from time to time. The actual schedule of doses is shown in Table II.

The doses of penicillin were quite small, approximately 160 units per day for a rabbit of 2300 gm., or about 70 units per kg. per day. The

dose of thiobismol was relatively larger, about 1.7 per kg. daily, approaching the upper limit of continuous doses for a human patient.

At necropsy the mitral leaflets were rough and thickened and there were distinct nodules on the posterior flap. The tricuspid leaflets appeared very slightly thickened. The aortic cusps were definitely stiffer than normal. The lungs were congested and there was extravasation of blood in the right lung. A few lesions of coccidiosis were seen in the liver. The spleen was somewhat enlarged, 55 by 10 by 4 mm., and the kidneys were grossly normal.

Microscopical study of the mitral valve revealed irregular angulation and distortion together with nodular fibrous thickenings attaining a diameter of 1 mm. The fibrils and cells of the valve were somewhat irregular in their arrangement and a few polynuclear leukocytes and lymphocytes were included in the stroma. Adhesions between adjacent folds of the leaflets had resulted in the formation of included vascular spaces lined by endothelium and containing blood. Continuous with these spaces there were narrower channels presenting the appearance of venous capillaries filled with blood. Apparently these new vessels communicated with the cavity of the heart and not with the coronary circulation. Throughout the thickness of the mitral leaflet there were clumps of yellowish brown pigment, much of it contained within phagocytic cells. This pigment was evidently derived from erythrocytes which had entered the stroma through defects in the endothelial covering of the valve during the period of active inflammation.

A portion of deformed mitral leaflet is depicted in Figure 19 and in greater detail in the colored drawing, Figure 21. The granules of brown pigment, chiefly contained within phagocytic cells in the interior of the structure, and a newly formed vascular space within the valve can be distinguished. A healed vegetation on the pulmonary valve of this animal is shown in Figure 20.

Rabbit 370, series 9B, inoculated with Streptococcus viridans (salivarius), strain F 345-8, daily for 18 days; early systolic murmur; treatment with penicillin; death on the 22nd day; healed mitral and aortic endocarditis; healed pulmonary endarteritis.

Rabbit 370, series 9B, weighed 1625 gm. on October 4. Auscultation of the heart was negative on October 7 and 11. *Streptococcus salivarius,* strain F 345-8, was given intravenously in doses of 0.125 to 0.250 cc. of bacterial suspension daily on October 10 to 27, inclusive. The temperature was 102.0° F. on October 9 and rose on October 11 to 107° in the morning and 106.8° in the afternoon. More remarkable was the appearance of a loud systolic blowing murmur first heard on the morning of October 12. A diagnosis of early mitral vegetative

endocarditis was confidently accepted. The murmur was much the same on October 13 but on October 14 the heart sounds were weak and the murmur was not recognized. Weak sounds were noted on October 16 and 20 but the murmur was not again detected. Cultures of the blood taken on October 12, 13 and 17 gave positive growth; those taken on October 24 and 27 remained negative. Until October 24 the animal's temperature was irregular and frequently reached high

TABLE III

Rabbit 370, Series 9B

Treatment with penicillin by intravenous (V) or intramuscular (M) injections. Clock time is shown on the basis of 24 hours, starting with zero at midnight. Doses are expressed in hundreds of (Oxford) units of penicillin.

Hour	0	2	4	6	8	10	12	14	16	18	20	22
Oct. 12											10-V	5-V
13	5-V	5-V	5-V	5-V	5-V	5-V	5-V	5-V	5-V	5-V	5-V	5-V
14	5-V	5-V	5-V	5-V	20-V	20-V	20-V	20-V	20-V	20-V	20-V	20-V
15	20-V	20-V	20-V	20-V	20-V	20-V	20-V	20-V	20-V	20-V	20-V	20-V
16	20-V	20-V	20-V	20-V	20-V	20-V	20-V	20-V	20-V	20-V	20-V	20-M
17	20-M	20-M	20-M	20-M	20-V	20-V	20-M	20-V	20-V	20-V	20-V	20-V
18	20-V	20-V	20-V	20-V	20-V	20-V	20-V	20-V	20-V	20-M	20-V	20-V
19	20-V	20-V	20-V	20-V	20-V	20-V	20-V	20-V	20-V	20-V	20-V	-
20	20-V	20-V	20-V	20-V	20-V	20-V	20-V	20-V	20-V	20-V	20-V	20-V
21	20-V	20-V	20-V	20-V	20-V	20-V	20-V	20-V	20-V	20-V	20-V	20-V
22	20-V	20-V	20-V	20-V	20-V	20-V	20-V	20-V	20-V	20-V	20-V	20-V
23	20-M	20-M	20-M	20-M	20-M	20-M	20-V	20-V	20-V	20-V	20-V	20-V
24	20-M	-	-	-	20-M	20-M	20-M	20-V	20-M	20-M	20-M	20-M
25	20-M	20-M	20-M	20-M	20-M	20-V	20-M	20-M	20-M	20-M	20-M	20-M
26	20-M	20-M	20-M	20-M	20-M	20-V	20-M	20-M	20-M	20-M	20-M	20-M
27	20-M	20-M	20-M	20-M	20-M	10-V	10-M	10-M	10-M	10-M	10-M	10-M
28	10-M	10-M	10-M	10-M			Died at 7:30 A.M., October 28					

levels; *i.e.*, 107.2,° 106.4,° 106.0,° 105.8,° 105.6,° 105.0,° but after October 24 the range was 101.2° to 104.0.° The weight of the rabbit diminished from the original 1625 gm. to 1615 gm. on October 9, 1585 gm. on October 15, 1440 gm. on October 23 and 1200 gm. on October 27. Death took place at 7:30 A.M. on October 28 and the body was at once fixed by arterial perfusion.

Treatment with penicillin alone was initiated on October 12 in an attempt to overcome the clearly evident early mitral endocarditis by a thorough course of penicillin in large doses, administered by intravenous or intramuscular injection as shown in Table III. The amount of penicillin used in the 17 days was 282,500 Oxford units, of which 176,500 were given intravenously and 106,000 by intramuscular injection. As the animal weighed 1625 gm., the penicillin used would corre-

spond to about 8.5 million units for a person weighing 50 kg., apparently an excessive amount.

At necropsy the posterior leaflet of the mitral valve appeared slightly thickened and the other valves were regarded as grossly normal. There were scattered lesions of coccidiosis in the liver. The spleen measured only 30 by 5 by 2 mm. before perfusion and 40 by 7 by 2 mm. after perfusion. The lungs and kidneys were grossly normal.

Microscopical sections through the mitral leaflets revealed a very marked thickening near the free margin and adjacent to the attachments of the tendinous cords. The deformity evidently had resulted from an inflammatory lesion but the precise character and extent of this lesion were no longer recognizable. In the substance of the leaflets there were very few wandering cells and only occasional red blood cells. Healing had evidently been complete for some time. The aortic leaflets also were irregularly thickened. In the lungs of this animal there were nodular thickenings in the walls of the smaller pulmonary arteries in which multinucleated giant cells and clefts indicating cholesterol deposits were present. The healed state of these pulmonary arterial lesions corresponded to the healed state of the cardiac valves.

A section through the deformed mitral leaflet is illustrated in Figure 22 and a section of the aortic valve in Figure 23.

Discussion and Comment

From observations on the course of experimental endocarditis lenta in rabbits and the study of the tissues after death, we have gained certain ideas in regard to the healing processes and the possible arrest of the disease in local lesions and in the entire animal. These observations seem to throw light on the course of events in the human disease and to promote a better understanding of the difficulties which confront the physician in his attempt to bring about an arrest of bacterial endocarditis and healing of the local lesions in his patient, and to some extent point the way to overcome these difficulties.

Relief of local stress at the site of the lesions is a primary indication and this may be favored by strict rest in bed and promotion of emotional calm. Intelligent, sympathetic nursing and freedom from annoyance by visitors and by other patients are desirable. General measures to maintain bodily resistance at a high level are of value. However, it must be evident that these measures will usually prove to be inadequate, once the vegetations with their bacterial colonies in necrotic fibrinous deposits have become well established. An increase of antibacterial agents in the blood is greatly to be desired. This increased concentration of these agents should be maintained for a pro-

longed period so as to restrain the multiplication of the streptococci, hamper the elaboration of their poisons and thus permit more effective eradication by bacteriolytic ferments and the intimate approach of phagocytic cells to engulf and digest them. It must also permit the successful proliferation of fibroblastic and endothelial cells in and on the fibrinous deposits to bring about the encapsulation of the infected material and its eventual organization into sterile scar tissue. Fortunately, there exist antibacterial agents which are known to exhibit a restraint upon the growth of the streptococci in the culture tube. These are the agents which we have attempted to select for experimental test in the diseased rabbits. Anti-infectious agents of promising potency in the culture tube may be expected, in many instances, to fail in the living animal because the conditions in the cardiac vegetation may be less favorable to their action and because the anti-infectious agents themselves may exert deleterious influences upon the tissues of the animal. The experimental therapeutic tests in the animals, therefore, may supply information to supplement the results of observations in the culture tube and thus aid in the selection of those anti-infectious agents most potent in the animal body and relatively free from deleterious effects, and these would appear to offer greatest promise for clinical trial in the human patient. The animal experiments may also aid in determining the most promising detailed program of therapeutic application of these agents, a matter of some importance.

In this communication we have attempted to present some of the results obtained in a prolonged study, in which many difficulties have been encountered. We are convinced that local healing of infected valvular vegetations and complete arrest of the disease process can be achieved in experimental endocarditis lenta of the rabbit. The therapeutic agents of greatest value include, among the synthetic chemicals, thiobismol, neoarsphenamine, mapharsen, sulfadiazine and sulfapyridine and, in the biological group, penicillin and bacteriophages. In our experiments the favorable effect of penicillin in experimental endocarditis due to *Streptococcus salivarius* has been conspicuous. In the experimental disease produced by inoculation with *Streptococcus faecalis* (enterococcus) the specific bacteriophages, in combination with thiobismol or neoarsphenamine, have seemed more promising. The chemotherapeutic agents alone have been less effective but we believe that they may exert a favorable influence.

The experimental therapy has, of course, already been extended to human patients by others [4-7] as well as by ourselves.[8-11] Here, as in the rabbit, we believe that the prolonged maintenance of a mild bacteriostatic concentration of the antibacterial agents in the circulating blood,

so as to restrain active bacterial growth at the site of the lesions, is the important desideratum. This favors local healing and at the same time tends to protect the distant organs from embolic insults. Complete arrest of the disease is in some respects similar to arrest in pulmonary tuberculosis. One cannot appraise with certainty the exact state of the completely or partially healed internal lesions. Prognosis in the arrested stage should, therefore, be guarded.

SUMMARY

1. Defense against experimental infection with streptococci of the viridans group brings into play a variety of mechanisms.

2. In the initial stage of bacteremic invasion the microbes are abundantly phagocytosed by the wandering leukocytes and especially by the fixed endothelial cells of the smaller blood vessels in general and to a less extent by the endocardial endothelium, and they are largely exterminated by digestion within these phagocytes.

3. After a bacterial vegetation has become established on a valve of the heart, the streptococci thrown off from it into the blood stream are largely disposed of by a similar process of phagocytosis. However, the bacterial colonies in the fibrinous vegetation, by their active growth and elaboration of toxic substances, ordinarily keep the host cells at a distance and continue to flourish.

4. To conquer these local bacterial nests, the first requirement is restraint of their active growth, which is not readily accomplished by the unaided defense mechanisms of the host. Second in importance appears to be the deposit of bacteria-free fibrin over the surface of the infected lesion, to serve as a framework for leukocytes, fibroblasts and endothelial cells, which come in to ingest the bacteria and organize the scar.

5. Restraint of bacterial proliferation is exercised not alone by antibacterial substances in the normal blood plasma and substances produced by the bacteria themselves, but may evidently be favored in a significant manner by chemical and biological therapeutic agents introduced into the body from without.

6. Local healing and complete general arrest of the experimental disease have been sometimes accomplished in rabbits and there is good reason to believe that similar success may be attained in the human disease.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 41

FIG. 1. Photograph of the left side of the opened heart of rabbit 166, series 4B, which died on the 30th day after initial inoculation with *Streptococcus faecalis* and subsequent treatment with thiobismol and specific bacteriophage. Part of the large, ragged mitral vegetation is shown.

FIG. 2. Photomicrograph at low magnification of a section through the mitral vegetation of rabbit 166, series 4B, stained by the method of Brown and Brenn. The mass of fibrin containing bacterial colonies, wandering cells and many irregular spaces rests chiefly on the auricular surface of the much thickened and distorted mitral leaflet. The very dark spots near the surface of the vegetation and many of the darker gray spots in the interior represent colonies of streptococci. There is also considerable cellular exudate in and near the base of the swollen leaflet and also in parts of the fibrinous vegetation itself. The thick ventricular wall and adjacent papillary muscle are easily recognized for orientation.

1

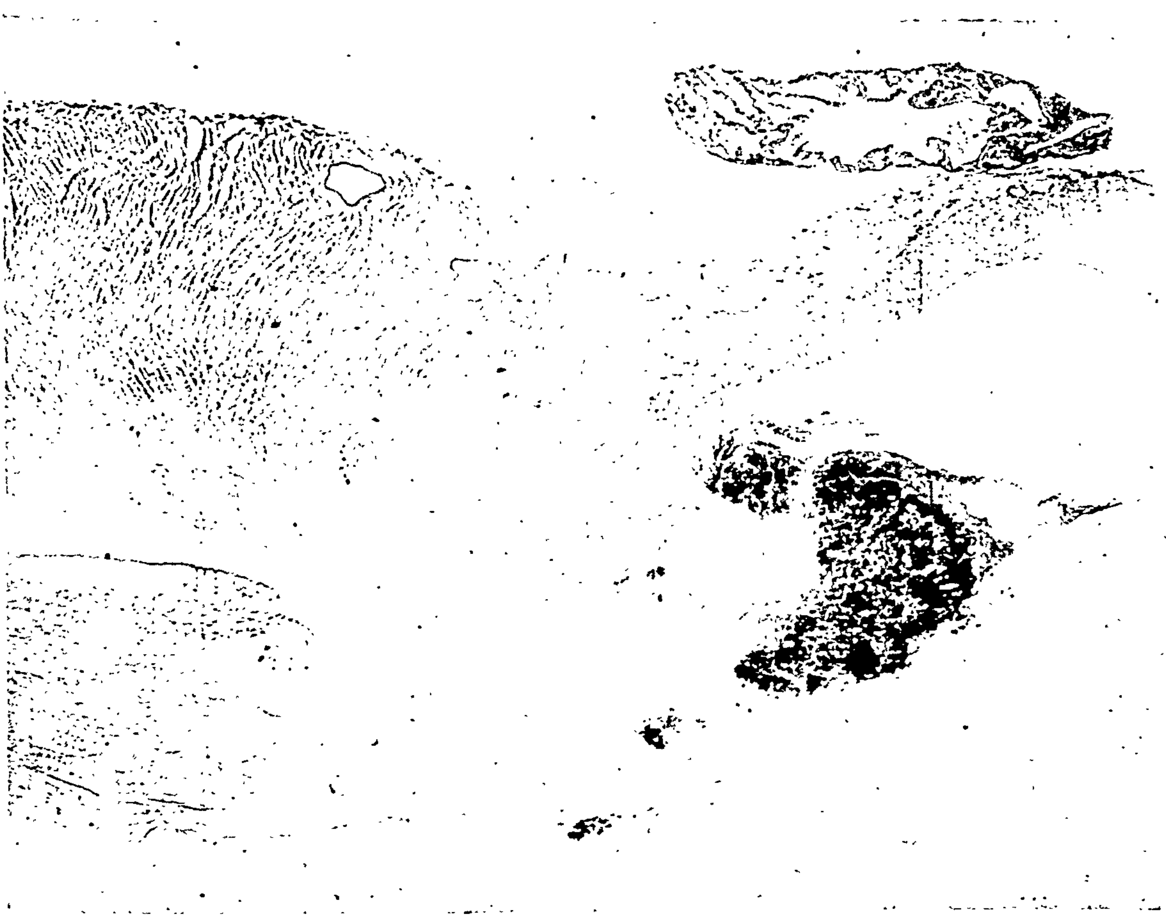

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Plate 42

Fig. 3. Photomicrograph showing more detail of the same vegetation. The bacterial colonies about the periphery retain a deep stain but they are for the most part covered over by a layer of fibrin, irregular in structure and thickness. The deeper bacterial colonies, on the other hand, show variable degrees of bacteriolysis, affecting especially the central portions of the colonies. At the midline of the figure and somewhat to the right of the center there is an irregular space containing at the left a dark-stained irregular clump of necrotic material. Below this cleft there are several bacterial colonies in which bacteriolysis is already far advanced. At the left near the lower corner is a large dark-stained mass of necrotic material and near this there is cellular exudate without well defined bacterial colonies. A small part of this exudate is represented in color in Figure 6.

Fig. 4. Photomicrograph of part of the mid-portion of the same vegetation. The irregular cleft with dark-stained necrotic clump is again recognizable to the left of the center of this figure. Above it, and to the left, are several bacterial colonies not in good focus, but showing nevertheless distinct central bacteriolysis. Left of the cleft is a group of three small bacterial colonies almost in contact with each other, which are shown in color in Figure 5. At their left is a colony largely destroyed by bacteriolysis and other large lytic colonies appear nearby. Below are smaller colonies, apparently confluent, with deeply stained cocci about the periphery. Cellular exudate is recognizable below and to the right but the bacteria present in this exudate cannot be distinctly seen at this magnification.

3

4

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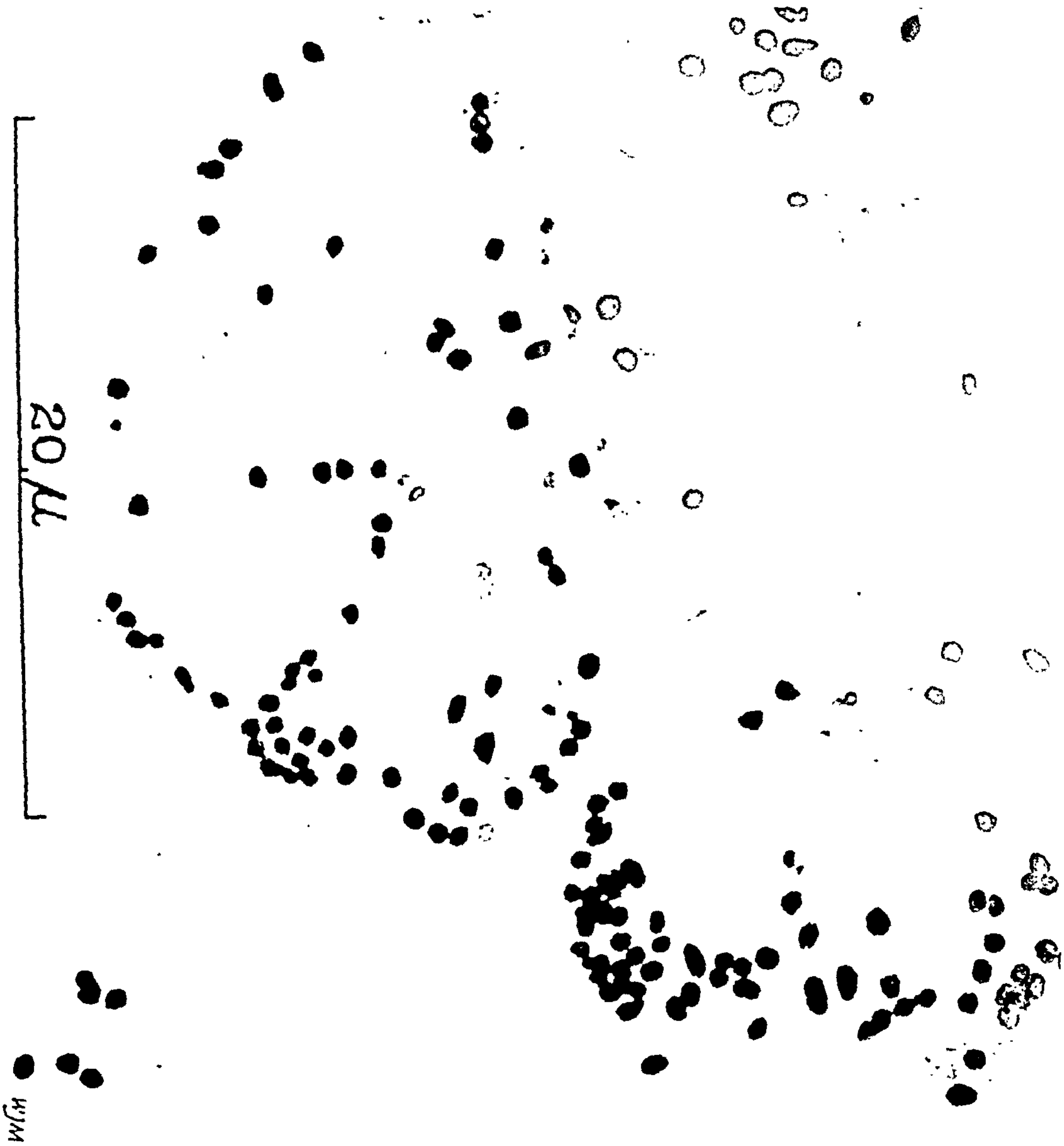
Plate 43

Fig. 5. Drawing at high magnification of the three bacterial colonies mentioned in the legend for Figure 4. The various stages of bacteriolysis and fragmentation of the streptococci can be recognized. Some of the bacteria still take the stain very well. Not a single phagocytic cell is present in the field of this drawing, nor is there any host cell present.

Fig. 6. Drawing of a small part of the cellular exudate mentioned in the legend of Figure 3. The exudate is full of deformed wandering cells adherent to each other and partly fragmented. They contain numerous phagocytosed cocci in various stages of growth and bacteriolysis. The streptococci are also lying free in the fluid serum. Apparently some agent or agents in solution have acted to permit these wandering cells to approach intimately the abundant bacterial groups and to engulf them.



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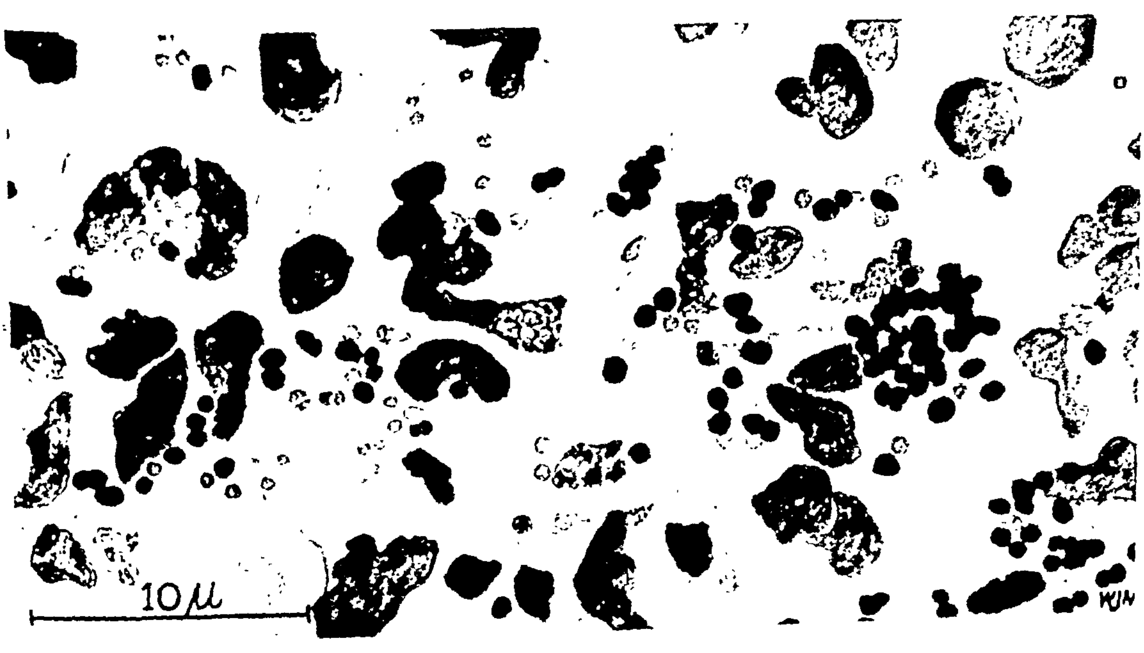

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Plate 45

Fig. 9. Drawing of the bacterial colony of Figure 8 with adjacent substance of the leaflet extending to the endothelial surface at the right. There are many individual bacteria scattered in the stroma even at some distance from the compact colony.

Fig. 10. Drawing, at high magnification, of the lower margin of the bacterial colony of Figure 8, showing stages of bacteriolysis at the periphery of the colony and phagocytosis of some of the streptococci in the neighborhood.

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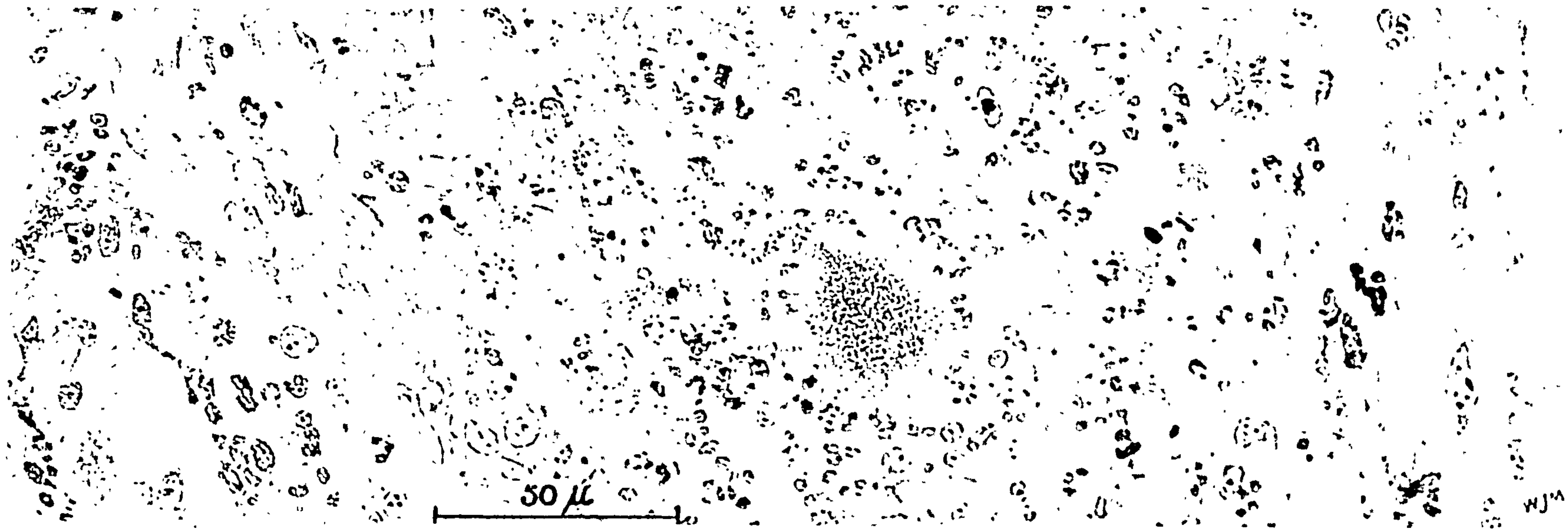

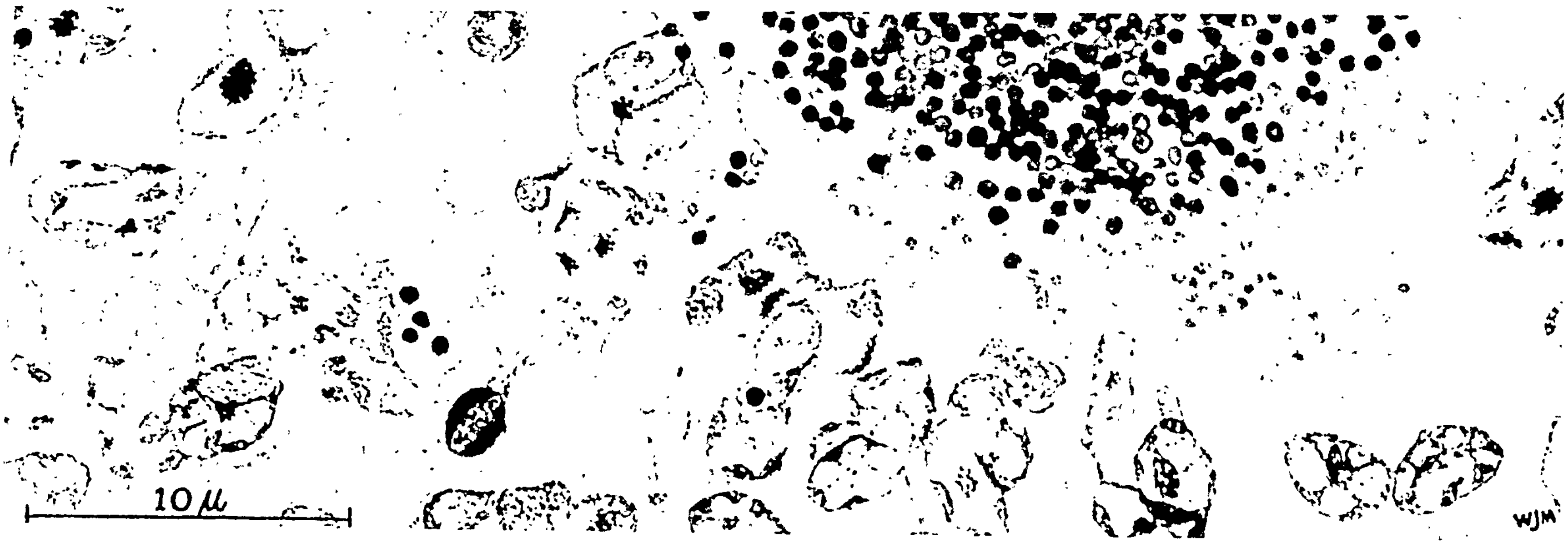

Plate 46

Fig. 11. Photomicrograph of a section of mitral leaflet of rabbit 593, series 14A, which died on the 18th day after initial intravenous inoculation, stained with hematoxylin and eosin. The vegetation is situated well out on the auricular face of the valve. There is an irregularly outlined center of fibrin, containing bacteria and wandering cells. Around this is a fairly complete capsule of fibroblastic tissue covered over with smooth endothelium.

Fig. 12. Drawing of a companion section of this same mitral leaflet of rabbit 593, stained by the method of Brown and Brenn, showing a small portion of the outer layers. The endothelial surface is at the right. The masses of bacteria lack the rounded contour of growing bacterial colonies and nucleated cells have been able to approach them and even to engulf many streptococci.

11

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PLATE 47

FIG. 13. Photograph of a gross section through the mitral ring of rabbit 392, series 5B, showing nodular deformity of the leaflets subsequent to intravenous inoculation with *Streptococcus viridans* and successful treatment with bacteriophage and thiobismol.

FIG. 14. Photomicrograph at low magnification of a section through the mitral ring of rabbit 133, series 2B, in which experimental endocarditis was evidently produced by intravenous inoculation and subsequently healed after treatment with bacteriophage and neoarsphenamine. The mitral leaflet is thickened and deformed.

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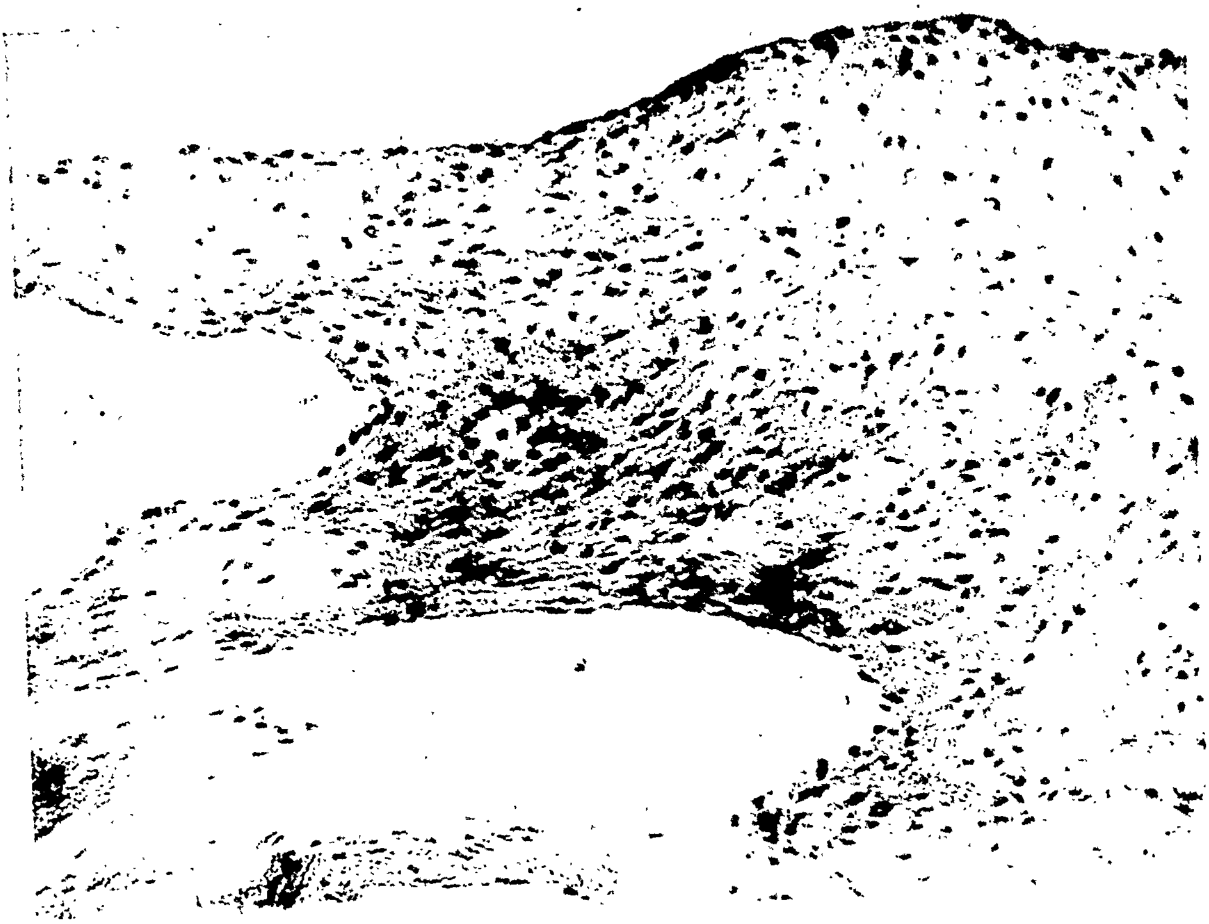
PLATE 48

FIG. 15. Photomicrograph of part of a companion section in this same series of sections through the mitral leaflet of rabbit 133, stained with hematoxylin and eosin, showing the irregular arrangement of cells and fibers in the thickened leaflet and, near the fork at the upper left of the figure, two diasters cut in a fortunate plane.

FIG. 16. Photomicrograph of an oblique section through the aortic ring of rabbit 133, series 2B, stained with hematoxylin and eosin. On the ventricular face of one of the leaflets there is an irregular fibrous projection shown in more detail in Figure 18.

5



6

PLATE 49

FIG. 17. Drawing of a small superficial portion of a section through a nodular thickening on the mitral leaflet shown in Figure 15, stained with hematoxylin and eosin. There is a mitotic figure in an endothelial cell at the lower right corner and two mitoses in fibroblasts to the left and toward the top of the drawing. The presence of a few lymphocytes and also the moderate edema suggest that extermination of the infectious agent may have been only recently accomplished.

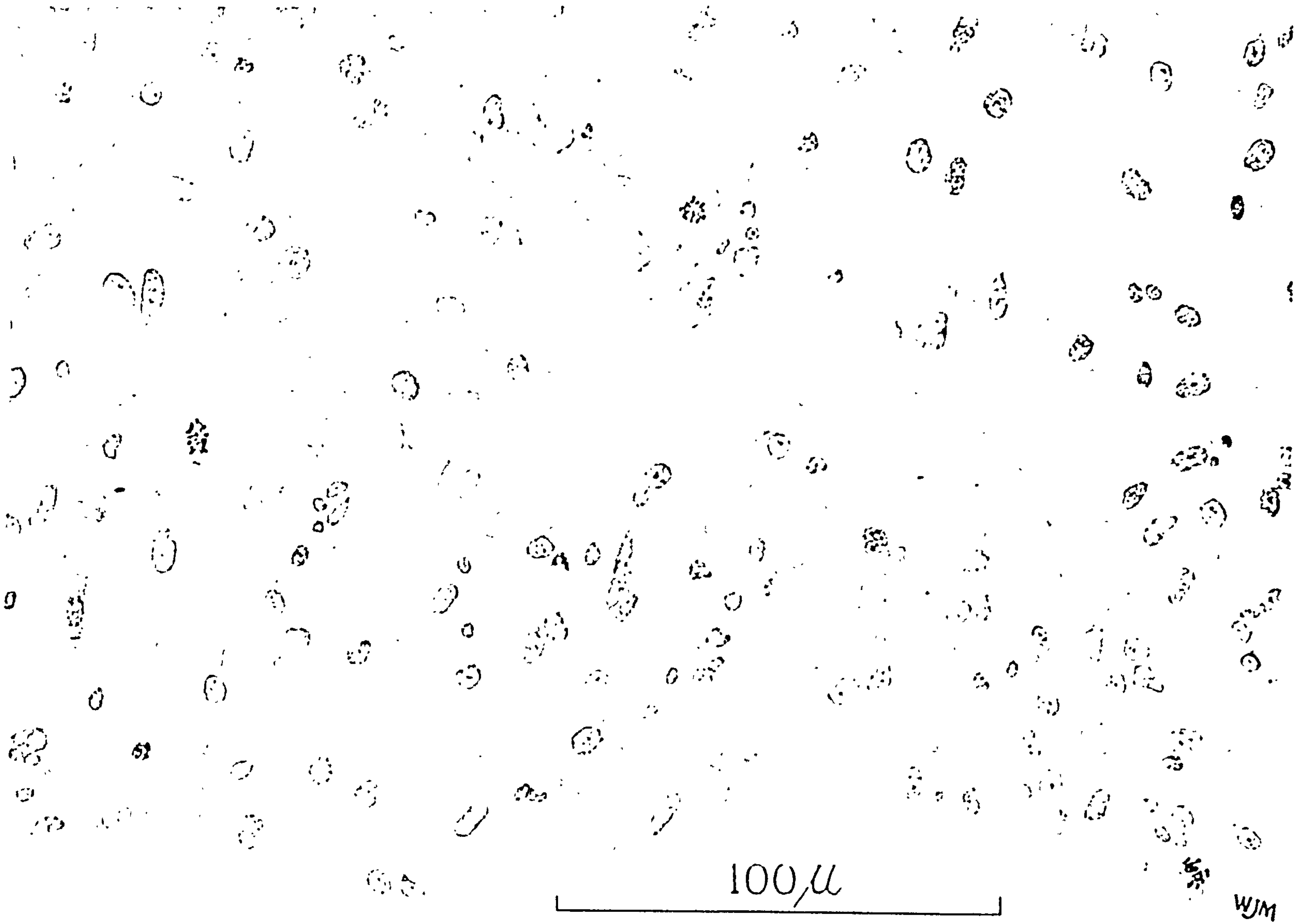


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Plate 50

Fig. 18. Drawing of the nodular prominence on the aortic valve seen in Figure 16. The nuclei of the endothelial cells are irregular in size and shape. The nodule is made up of fibroblasts and fibers with spaces less prominent than in the mitral nodule of Figure 17. There are, however, nuclear division figures, a few lymphocytes and one erythrocyte included in this section of the nodule.

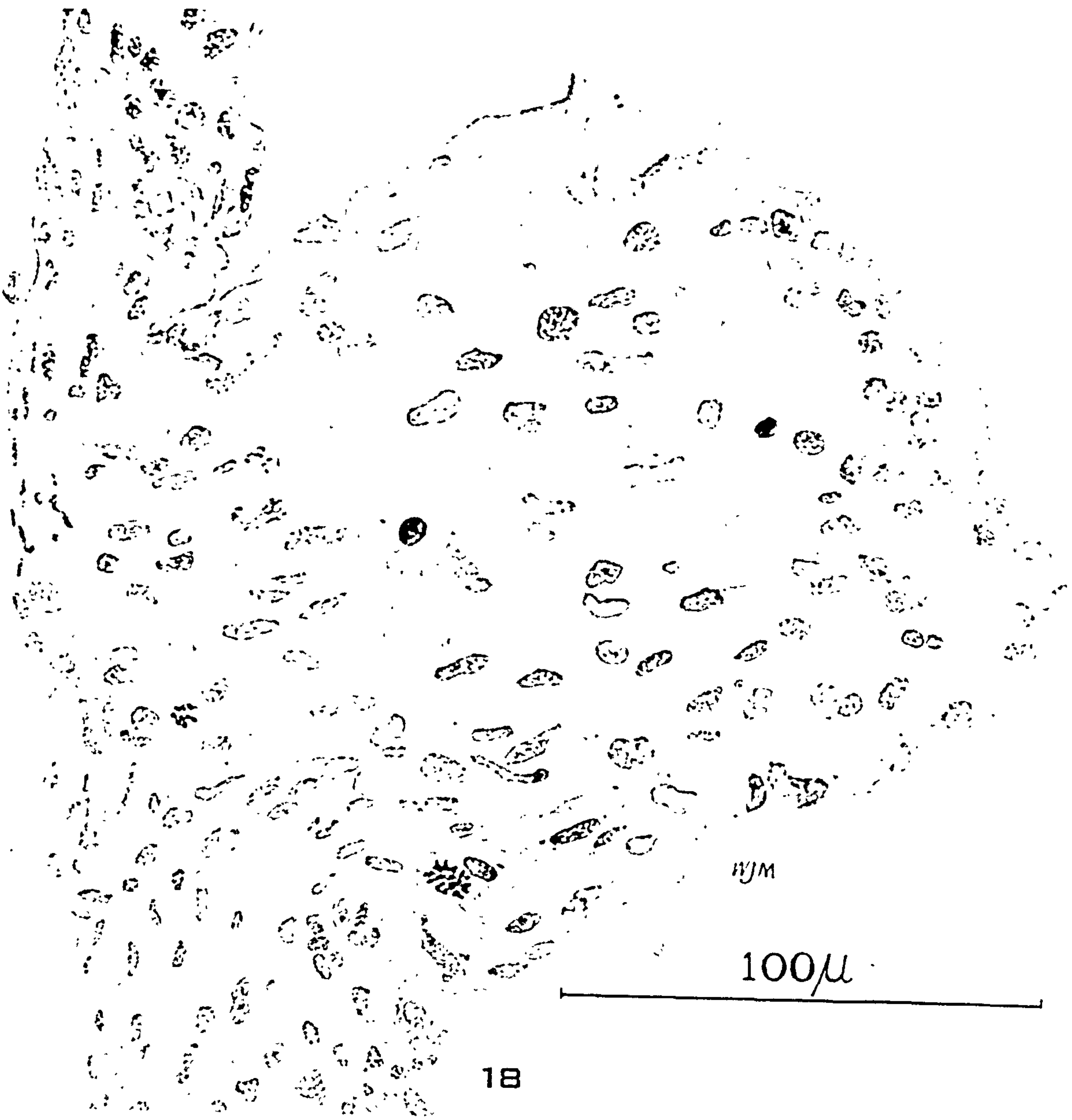

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PLATE 51

FIG. 10. Photomicrograph of a small portion of a section of the deformed mitral valve of rabbit 362, series 8B, stained with hematoxylin and eosin. This rabbit was inoculated intravenously with *Streptococcus salivarius* daily for 23 days and treated with penicillin and thiobismol. The endothelial cells are irregular in form, size and arrangement, and form several compact strata near the tip of the nodule, shown at the lower right. Above this region is an included vascular space or capillary, filled with blood. More detail is shown in Figure 21.

FIG. 20. Photomicrograph of a section stained with hematoxylin and eosin passing through a deformed pulmonary semilunar cusp of rabbit 362, series 8B. Apparently the irregular thickening represents a healed lesion of endocarditis on this valve.

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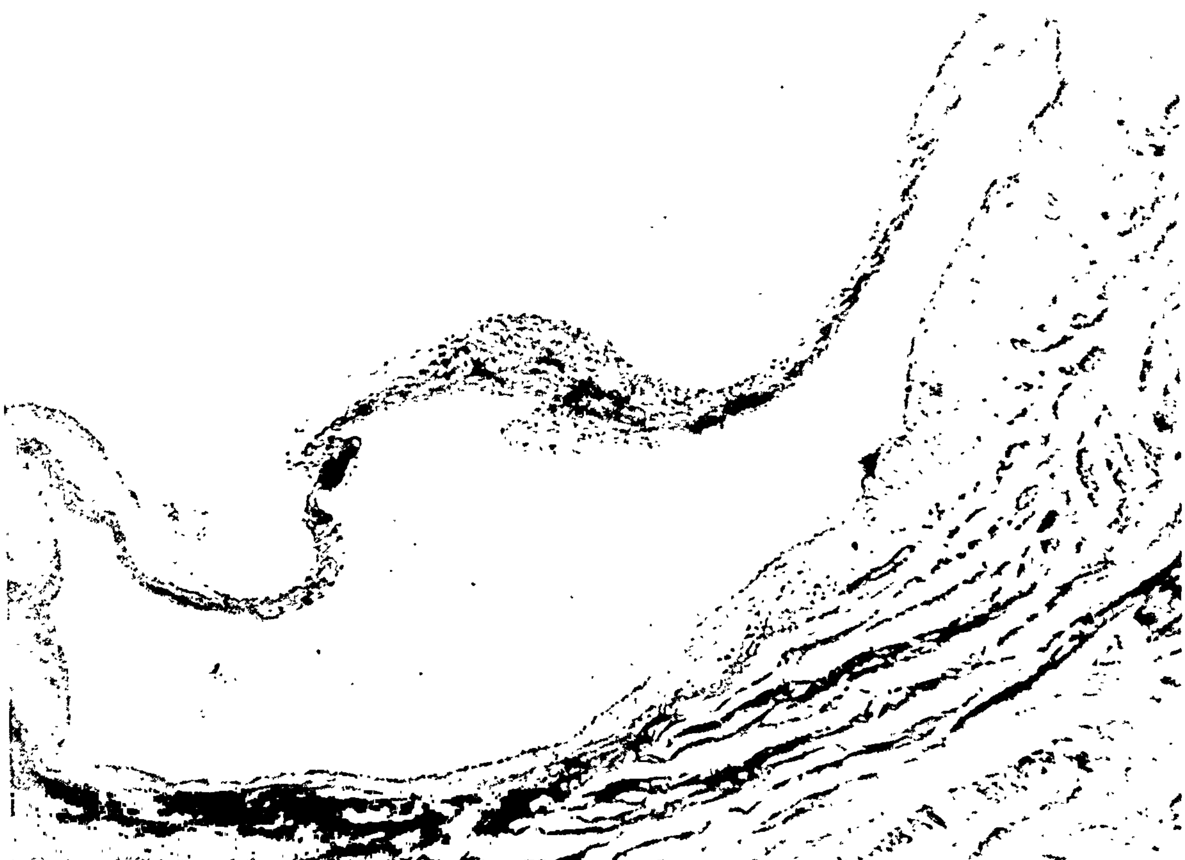

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PLATE 52

FIG. 21. Drawing of part of the deformed mitral leaflet of Figure 19. A group of brown granules at the extreme left of the central figure is represented at higher magnification (see scale below) in the upper left corner. A similar collection of granules at the right center of the large figure is shown on the larger scale in the upper right insert. A cell at the left center of the central figure is depicted on the larger scale as an insert at the lower right corner.

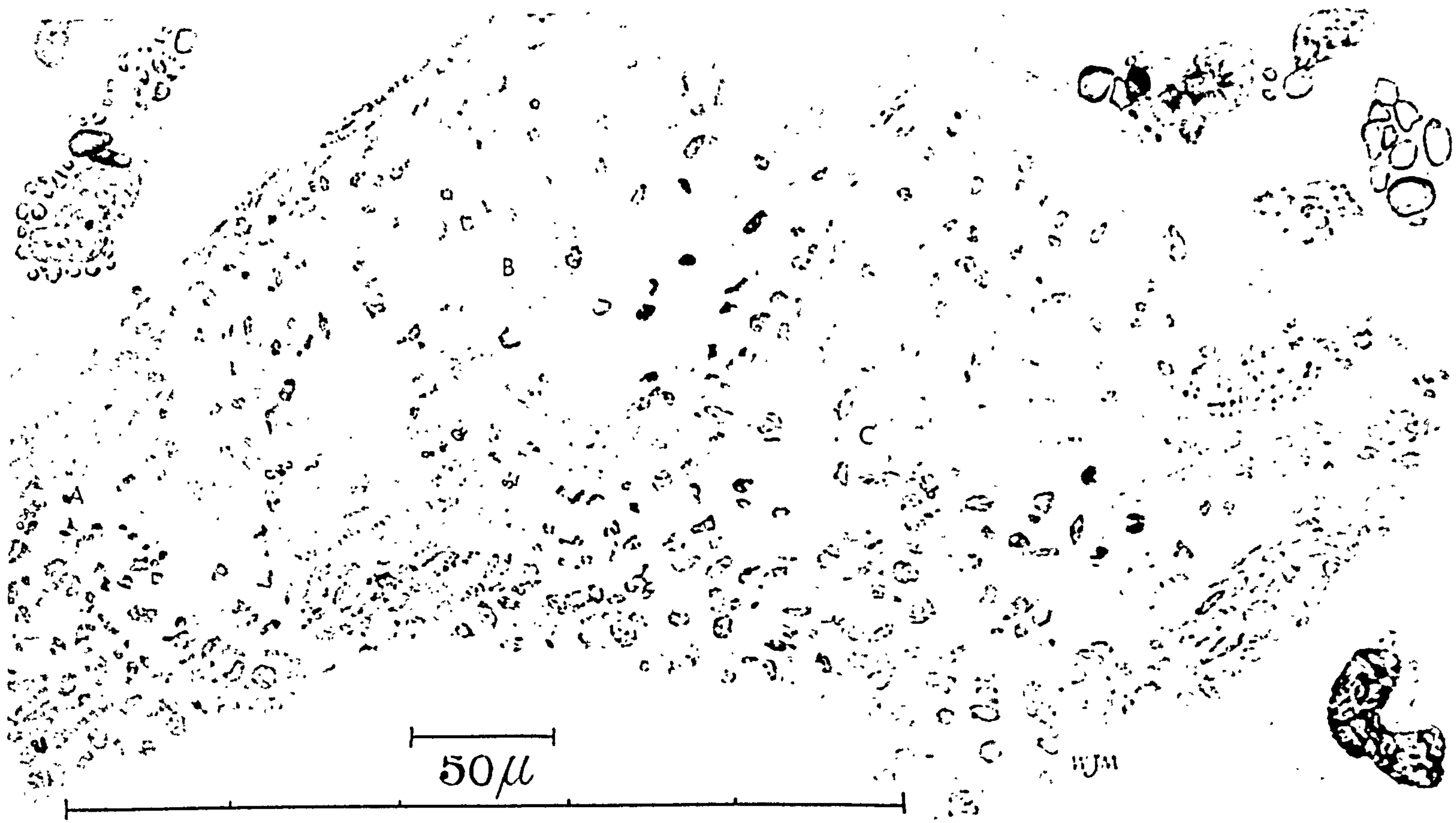

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PLATE 53

FIG. 22. Photomicrograph of a section stained with hematoxylin and eosin, through part of the mitral valve of rabbit 370, series 9B. This rabbit was inoculated with *Streptococcus salivarius,* developed a loud mitral murmur and high fever and was treated with large doses of penicillin. The mitral leaflets are thickened and deformed.

FIG. 23. Photomicrograph of a section stained with hematoxylin and eosin, passing obliquely through the aortic ring of rabbit 370. There is irregular thickening of the aortic cusps indicating a healed lesion of bacterial infection.

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Experimental Endocarditis Lenta

STUDIES ON TUMORS OF THE TESTIS

II. The Morphology of Testicular Tumors of Dogs *

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The testicular tumors of dogs are of special interest because of their frequency, the rarity of metastasis and often because of their production of androgen or estrogen. While several types of canine testicular tumors closely resemble the tumors of man, others do not have a counterpart in human pathology. This study is concerned with the morphology of 64 tumors of the testes of 41 dogs with special reference to the nontumorous testicular tissue, the prostate as an indicator of hormonal production and the extent of necrosis in the tumor.

Despite a complex terminology, most workers agree that the common testicular tumors in dogs may be classified into three groups: (1) interstitial cell tumors; (2) seminomas (embryonal carcinoma, grosszelligen Tumoren, seminal epithelioma); (3) tubular adenomas (adenocarcinoma, Sertoli cell tumors). Early reports of isolated cases of canine testicular tumors have been recently reviewed.[1,2] Kunze [3] observed 23 canine testicular tumors which were classified thus: 13 interstitial tumors, 5 seminomas, 4 adenomas, 1 spindle cell tumor. Pallaske [4] observed 38 tumors in the testes of 107 dogs; 31 were interstitial tumors and 7 were apparently derived from germinal epithelium. Peyron, Blanchard and Salomon [5] described 31 interstitial tumors and 5 seminomas in the testes of old dogs. An extensive series was reported by Schlotthauer, McDonald and Bollman [1] who studied 82 spontaneous tumors in 59 testes of 48 dogs without evident metastases; there were 51 interstitial cell tumors, 25 seminomas and 6 adenocarcinomas. The same group [6] analyzed the seminomas in detail—7 of them were diffuse while 18 seminomas were wholly or partly confined to the tubules; the latter observation supported the idea of Chevassu [7] that seminomas arise from epithelium of seminiferous tubules. Innes [2] reported on 49 canine testicular tumors which he classified as follows: 32 seminomas, 15 Sertoli cell tumors, 2 malignant interstitial cell tumors. Metastases to the periaortic lymph nodes were associated with 1 interstitial cell tumor, with uncertain evidence of metastases in the second case; in 1 seminoma there was evidence of metastasis on microscopic examination of a lymph node. These are the only observations in the literature of metastases of canine testicular tumors. There was nodular hyperplasia

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of the interstitial cells in 12 cases which Innes did not regard as neoplastic.

The association of squamous metaplasia of the prostate with canine testicular tumors is of interest; this estrogenic stimulation is accompanied by other evidence of femaleness. Greulich and Burford [8] reported the findings in 3 dogs which had tumors located in cryptorchid testes with replacement of the cylindrical epithelium of the prostate with squamous cells; 2 of these dogs were sexually attractive to other males and had hypertrophy of the mammary papillae. The same syndrome has been reported by Zuckerman and co-workers.[9, 10] In an autopsy series of 243 dogs, 35 testicular tumors were observed: [9] seminomas, 17; adenocarcinomas, 15; interstitial cell tumors, 3. In 5 dogs there was estrogenic stimulation of the prostate, all associated with adenocarcinoma.

The experimental production of interstitial cell tumors in certain strains of mice by estrogenic administration was accomplished by Bonser and Robson [11] and by Hooker, Gardner and Pfeiffer.[12] These tumors are both benign, and malignant, metastasizing to regional lymph nodes, lung and above the kidney.[11,13,14] In this case estrogen produces atrophy of the involved testis to the Sertoli cell stage, with spermatogonia and occasionally spermatocytes being present.[11,12]

Methods

The high incidence of tumors in aged dogs has been repeatedly observed.[15-17] The tumors in the present study occurred without exception in very old animals; evidence of senility was the occurrence of lenticular opacities and wearing and discoloration of the teeth, especially of the incisors. The dogs were killed by electric current and the testes and prostates were weighed and then fixed in 10 per cent formalin. Frozen sections were made with staining for fat with sudan IV; paraffin sections were stained with hematoxylin and eosin. Chemical analyses were made on 23 of these tumors [18] and the results are correlated with the morphologic appearance in the present paper.

Results

No teratomas, sarcomas, or chorionic epitheliomas were found. All of the canine tumors were epithelial in nature and could be classified easily into four groups: interstitial cell tumors, seminomas, tubular adenomas and undifferentiated carcinomas. A statistical analysis is presented in Table I. All of the tumors were very vascular and it is known that such tumors bleed profusely when incised for biopsy.

Interstitial Cell Tumors

Most of the interstitial cell tumors were small, commonly 1 to 3 cm. in diameter, and consisted of a single nodule, yellow-orange, and of a consistency resembling brain. The weight varied from 1.87 to 24 gm. The nodules were discrete and were separated from the surrounding testis by a connective tissue capsule, several fibroblasts in thickness.

TABLE I

Statistics of Testicular Tumors of Dogs

Number of dogs with testicular tumors:					41
Number of testes involved:					58
Number of tumors:					64
Bilateral tumors: 17		Unilateral: 24			
Number of interstitial cell tumors:					33
Number of seminomas:					19
Number of tubular adenomas:					9
Number of undifferentiated carcinoma:					3
Multiple tumors in same testis:					6
Interstitial cell tumor and seminoma:					5
Interstitial cell and tubular adenoma:					1
State of germinal epithelium of the uninvolved testis:					
Interstitial cell tumor					
Normal: 11	Atrophy: 5				
Seminoma					
Normal: 5	Atrophy: 2				
Tubular adenoma					
Normal: 3	Atrophy: 2				
Undifferentiated carcinoma					
Normal: 3	Atrophy: 0				
State of the prostate:					
Interstitial cell tumor					
Normal: 3	Cystic hyperplasia: 18		Atrophy: 0		
Seminoma					
Normal: 2	Cystic hyperplasia: 7		Atrophy: 1		
Tubular adenoma					
Normal: 0	Cystic hyperplasia: 1		Atrophy: 4		
Undifferentiated carcinoma					
	Cystic hyperplasia: 2		Squamous metaplasia: 1		

On microscopy, it was seen that the cytoplasm was relatively much larger than the nucleus. The nucleus was basophilic and varied in size, sometimes small and dense, at other times larger and paler; it contained a single large nucleolus. The nucleus was always eccentric and usually polar. The cytoplasm was extensive, acidophilic and, containing many fat droplets, appeared foamy with vacuoles in a defatted section. Many of these tumors consisted of solid masses of cells, but in others, in addition, anastomosing cords often draped with macrophages

were prominent. These phagocytes laden with brown pigment were usually seen in this tumor. Areas of necrosis were common, with regions completely replaced by fat or hemorrhage or connective tissue. Viable areas of interstitial cells were always found and in no tumor was necrosis complete.

Seminomas

The seminomas were usually larger than interstitial cell tumors and their weight varied from 8 to 40 gm. In the gross they were white, firm and multinodular.

On microscopic examination a single type of tumor cell was found occurring either diffusely or confined to the seminal tubules; both the diffuse and tubular forms often occurred in the same testis. Among the 19 seminomas, 9 were diffuse, 5 tubular, 4 were both diffuse and tubular and 1 testis was nearly completely necrotic. The cell consisted of a single, large, basophilic nucleus with a small amount of weakly basophilic cytoplasm. The cells of canine and human seminomas resemble each other except that the canine tumor is more basophilic; the collections of lymphocytes which always accompany human seminomas are absent in the tumors of dogs.

Necrosis is less common in seminomas than in interstitial cell tumors. In the series of 19 seminomas, 16 were free from necrosis, 1 tumor was almost completely necrotic, another had extensive hemorrhage and a third had a nonlipoid type of vacuolated degeneration.

Tubular Adenomas

In the group of tubular adenomas the largest tumors occurred, weighing 4 to 408 gm. In the gross these tumors were white, solid, coarsely nodular and free from necrosis. They were bilateral in 4 dogs, in 1 of which the tumors were very large and situated in cryptorchid testes; a unilateral tumor was found in 1 dog.

Microscopically, tubular adenomas were found to consist of many closely packed tubules free from cyst formation. The tumors were not accompanied by Leydig cells and the stroma was sparse; they did not infiltrate but rather displaced the normal testicular tissue. The cells were long and irregular in shape and terminated in thread-like processes. In many instance the cells filled the tubules, extending from side to side and growing and clumping in the lumina. The cytoplasm was acidophilic and the nucleus was weakly basophilic.

Perhaps the most favorable situation in which to study normal Sertoli cells in the adult is in the testis of cryptorchidism. Here the tubules are not dilated and are separated by large groups of interstitial cells. The Sertoli cells are aligned along the basement membrane and

their cytoplasmic fibrils anastomose in the lumen. The nucleus of the Sertoli cells is strictly basal, arranged along the tubular basement membrane. In contrast to the normal Sertoli cells of undescended testes, the cells of tubular adenomas have lost their basal orientation in growing into the lumina of the tubules and there are no intratubular masses of Leydig cells; moreover, the nucleus is larger and less dense. Otherwise there is a close similarity of Sertoli cells of the cryptorchid to the principal cell of the tubular adenoma.

Undifferentiated Carcinoma

The group of undifferentiated carcinomas was small and consisted of 3 tumors, in which it was not readily possible to classify the carcinomatous cells with reference to the architecture of the normal testes. In 2 cases the tumors were large, weighing 24 and 47 gm., their cells resembling interstitial cells but with a low fat content. In both instances there was an associated senile cystic hyperplasia of the prostate. In a third case the tumor weighed 28 gm.; there was a diffuse pattern of large cells with a high fat content but the cells did not resemble interstitial cells nor were they arranged in tubules. This tumor was of interest since it was accompanied by estrogenic stimulation of the prostate, manifested by replacement of the cylindrical epithelium by multilayered squamous cells, many of which were lying free in the alveoli. The prostate weighed 11 gm., and projecting from one lateral surface there was a spheroidal bud 1.2 cm. in diameter.

The Fat Content of Testicular Tumors

The normal fat content of adult canine testes was found to be 1.47 ± 0.32 gm. per 100 gm. of wet weight.[18] On staining normal testes with sudan stain, fat was found in two types of cells, Leydig cells and Sertoli cells. Large amounts of fat were found in interstitial cell tumors (4 to 14.9 per cent) and in tubular adenomas (2.1 to 6.8 per cent) and this was reflected in the cells staining with sudan stain. The interstitial cell tumors were packed with fat droplets. There were free fat droplets in many of the lumina of the tubular adenomas as well as fat in the neoplastic cells. Also in the tubular adenomas, many of the tubules were surrounded by an areola of sudanophilic material in the peritubular connective tissue. Other tubules were free from fat in the lumina and cells, as well as in the surrounding areola. Seminomas did not stain with sudan dyes; their fat content varied from 0.2 to 0.6 gm. per cent. The undifferentiated tumor (fat content, 7.5 per cent), associated with estrogenic stimulation of the prostate, had masses of fat in the nuclei of the tumor cells.

Discussion

The determination of the neutral fat content of testicular tumors by chemical methods paralleled closely the staining with sudan dye. The sudan staining proved a useful aid in classification. The key is as follows:

1. Low fat content of cells—seminoma
2. High fat content with cells arranged in tubules—tubular adenoma
3. High fat content of vacuolated cells with absence of tubular arrangement—interstitial cell tumor

A single error was made in that an undifferentiated carcinoma of large cell type, producing estrogenic stimulation of the prostate, had a high fat content; there was absence of tubular arrangement and the cells did not resemble Leydig cells.

The general morphology of tubular adenomas, together with their high content of lipoids, prove that the neoplasms are tumors of Sertoli cells. In describing the sustentacular cells of the testis, Sertoli [19] discovered that these cells had a high fat content. Pick [20] first described a tumor of cylindrical epithelium, which he named "adenoma tubulare testiculare ovarii," in ectopic human testes and in the ovary of a pseudo-hermaphrodite. In the canine tubular adenomas the cells are larger, the alveoli smaller and more closely packed than in the Pick tumor in which the nuclei are basal. The cells do not grow into the lumina of the alveoli nor do they contain fat. Thus the similarity of these tumors is not close.

The large number of tumors with morphologic characteristics resembling cells of the normal testis with preservation of their normal lipoid relationships is evidence that the majority of the testicular tumors of dogs approach physiologic maturity—a point of significance from the standpoint of experimental and clinical cancer therapy. Moreover, none of the dogs with interstitial cell tumors had atrophy of the prostate, suggesting elaboration of androgens by the tumor; both tubular adenomas and seminomas were associated with prostatic atrophy (Table I) in several instances. The finding of a few large tumors with cells resembling interstitial cells but without fat is evidence that these tumors are physiologically more immature than the common type of interstitial cell tumors.

In the experimental production of interstitial cell neoplasm in mice by estrogen, moderate to severe atrophy occurred in the germinal epithelium of nontumorous regions of the testis. In two-thirds of the cases of spontaneous interstitial cell tumors the germinal epithelium was normal in appearance. In the undifferentiated carcinoma in this series which produced estrogen as manifested in squamous metaplasia

of the prostate, the germinal epithelium of the uninvolved regions of the testis was quite normal. This is evidence that the naturally occurring estrogens are different in kind or amount from those administered in the experimental production of interstitial cell tumors.

The close morphologic resemblance of canine and human seminomas suggests that these tumors have a comparable site of origin. The canine seminomas are much more frequently confined to tubules than the human tumors. The cell type in tubular and diffuse seminomas, however, is identical and these observations support the theory of Chevassu [7] that this tumor arises from germinal epithelium.

Summary

In a morphologic study of 64 tumors contained in 58 testes of 41 dogs, it was easily possible to classify the neoplasms in four groups: 33 interstitial cell tumors, 19 seminomas, 9 tubular adenomas, 3 undifferentiated tumors. Two of the undifferentiated tumors resembled interstitial cells but did not contain fat; a third tumor of this class had a high lipoid content but its cells did not resemble Leydig or Sertoli cells and was accompanied by squamous metaplasia of the prostate but with normal germinal epithelium in the testis. From a morphologic standpoint it may be inferred that most canine testicular tumors are physiologically mature; as further evidence, the prostates of all of the dogs with interstitial cell tumors were normal or enlarged while prostatic atrophy was observed in each of the other groups. Metastasis was not found in any case.

Staining of the tumors with sudan dyes proved useful in classification. Interstitial cell tumors had diffuse cells laden with fat. Many of the cells of tubular adenomas contained fat droplets; many of the alveoli were surrounded by a lipid areola. Seminomas contained no fat.

Evidence is presented that interstitial cell tumors are derived from Leydig cells, tubular adenomas from Sertoli cells and seminomas from germinal epithelium.

Moderate or severe atrophy of germinal epithelium has followed the experimental production of interstitial cell tumors with estrogen; two-thirds of the spontaneously developed interstitial cell tumors were accompanied by normal germinal epithelium.

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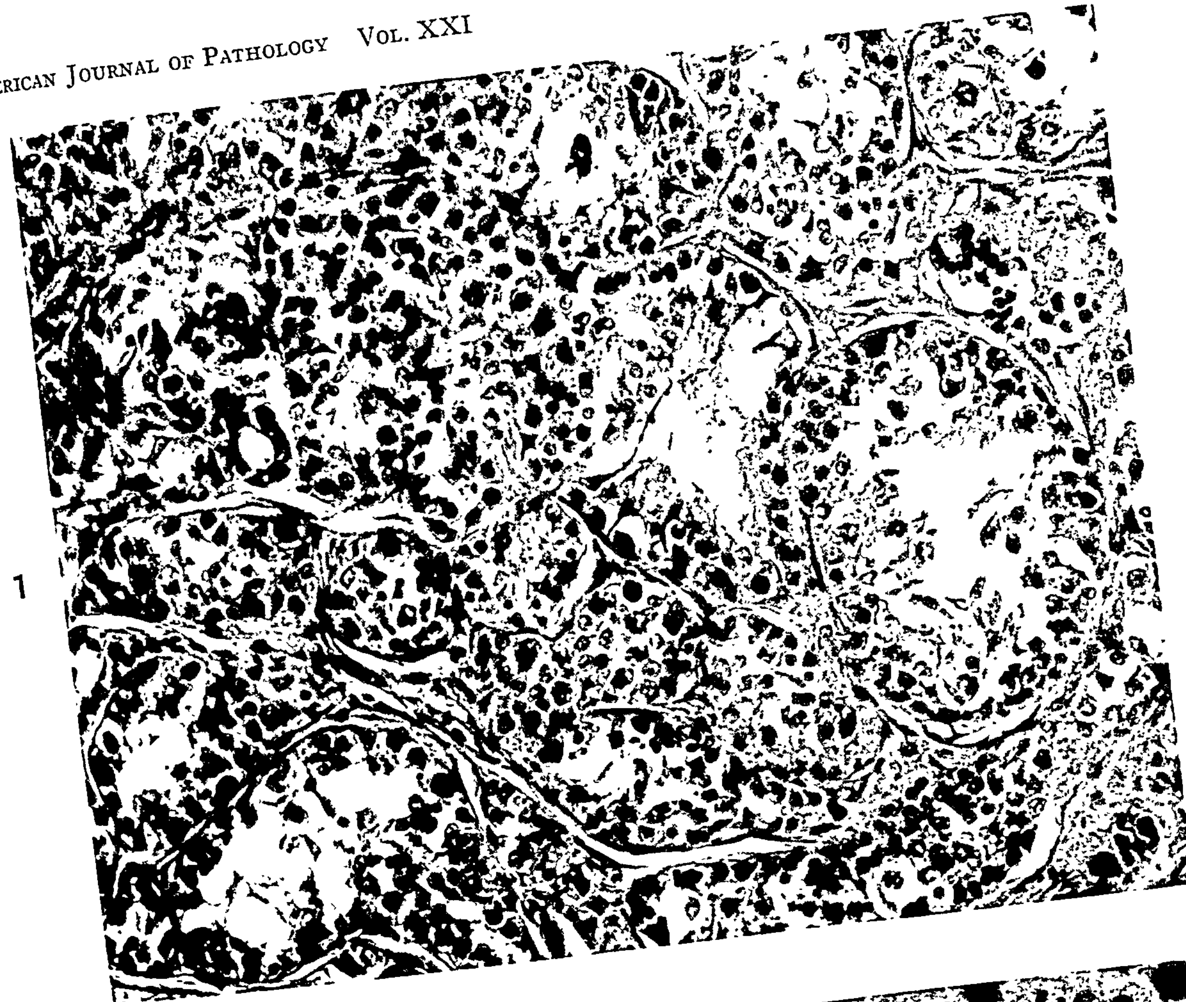
DESCRIPTION OF PLATES

PLATE 54

FIG. 1. Tubular adenoma of canine testis. × 250.

FIG. 2 Diffuse carcinoma of canine testis. × 475.



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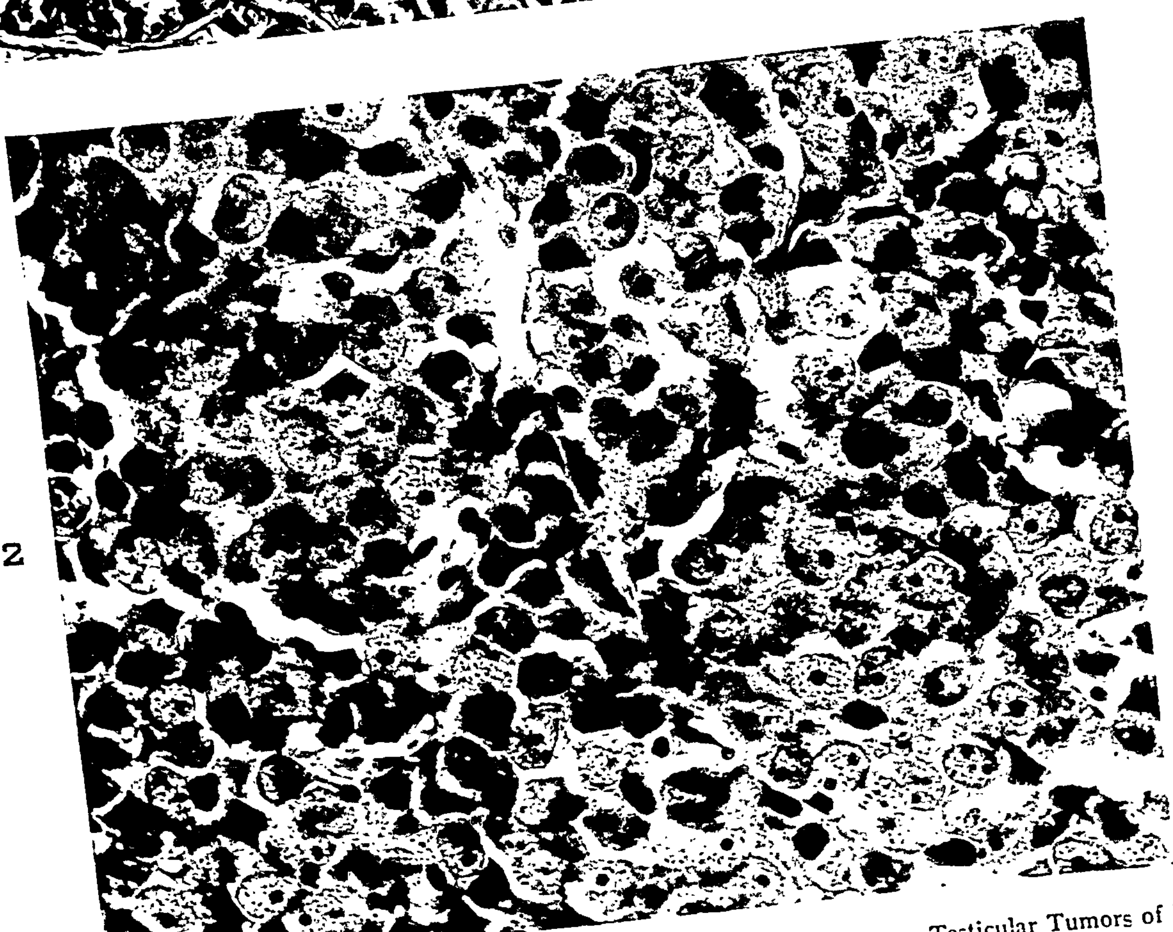


2

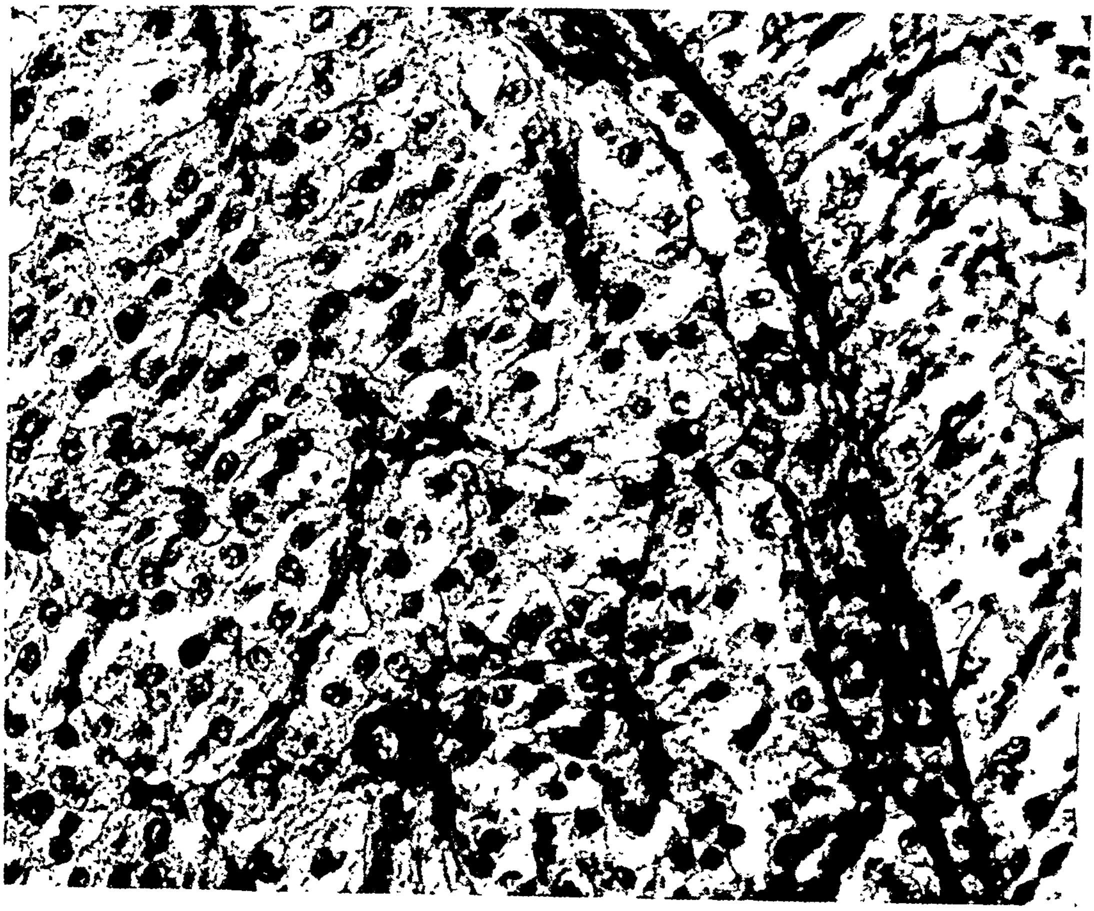
Testicular Tumors of Dogs

PLATE 55

FIG. 3. Interstitial cell tumor of canine testis; on the right is normal testicular tissue. × 520.

FIG. 4. Interstitial cell tumor and tubular seminoma of canine testis. × 160.

3



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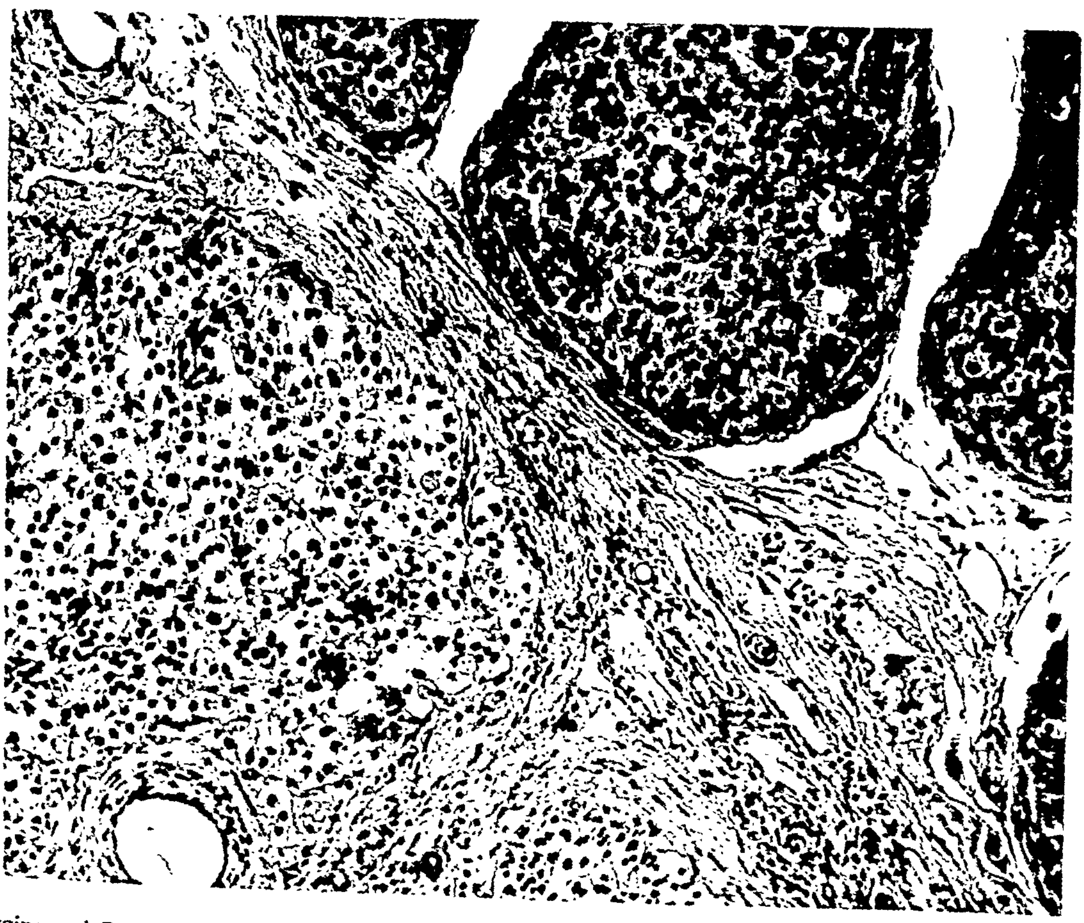

Huggins and Pazos

Testicular Tumors of Dogs

THE EFFECTS OF INHALED HEAT ON THE AIR PASSAGES AND LUNGS

An Experimental Investigation *

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Victims of conflagrations frequently sustain pulmonary injuries that are of equal or greater importance to survival than are the burns received on the surface of the body. In some instances the changes are confined to the upper air passages with little or no damage to the lungs. In some the larynx and trachea show little or no evidence of injury and profound pulmonary damage is sustained. In still others the entire tract is affected.

The precise cause of such respiratory injuries is usually obscure. In some conflagrations, as for example the Cleveland Clinic disaster in 1929, none of the victims was exposed to excessive heat and most of the deaths were believed to be due to the breathing of chemical irritants contained in the smoke that was given off by the burning of nitrocellulose x-ray films.[1] In many conflagrations, however, the victims have been exposed to heat as well as to smoke, and if respiratory injuries are sustained the question of whether they were due to thermal or to chemical trauma is likely to be raised.[2]

It is obvious that no two nonexperimental fires are likely to be exactly alike so far as their propensity for the elaboration of chemically irritating combustion products is concerned. A fire represents a series of chemical reactions, each of which may contribute a different group of potentially injurious gaseous, liquid, or solid irritants to the atmosphere. The nature and amount of these potentially injurious substances in smoke are determined in part by the composition of the material being burned, in part by the amount of oxygen that is available at different times during the conflagration, and in part by the temperatures that are attained. Among the more dangerous irritants likely to be encountered in smoke are aldehydes and acid anhydrides.

However numerous and variable the chemically injurious properties of smoke may be, it is apparent that the problem as a whole would be less formidable if a better understanding were had of the pathological characteristics of uncomplicated thermal injury of the lungs and air passages and of the temperatures that are necessary for their production. The opportunity to study such injuries in man in circumstances in which the possibility of concomitant chemical injury can be excluded is rare. It was decided, therefore, to investigate the problem under controlled conditions in the laboratory.

* Received for publication, November 20, 1944.

Experimental Methods

Eighteen dogs were used to investigate the effects of hot air on the respiratory tract and two pigs were used to acquire information regarding the relative vulnerability of the skin and the lining of the respiratory tract to certain types of exposure. All experiments were conducted under anesthesia induced by the intravenous or intraperitoneal injection of sodium pentathol.

In order to study thermal injuries of the lungs independently of any complications that might be caused by concomitant burning of the skin, the hot air was conveyed directly to the trachea by means of an insulated trans-oral cannula. The internal end of the cannula extended below the vocal folds of the larynx and the external end protruded from the mouth. In such experiments neither the skin nor the mucous membranes of the mouth or throat were exposed to heat.

Three types of inhalation experiments were performed. In the first the animals breathed ordinary hot air at different external temperatures. In the second the flame and combustion products from a blast burner were inhaled. In the third the animals breathed a mixture of live steam and air.

The external temperature of the air available for respiration in each type of experiment did not fluctuate to any considerable degree from breath to breath and was measured by a thermometer in some experiments and by a platinum-rhodium thermocouple in others. As the hot air was breathed the temperature within the internal air passages rose rapidly during inspiration and returned to, or almost to, normal during expiration. To measure these changes two thermocouples constructed of fine (36 or 40 gauge) wire were installed in the airway, one being placed where the inhaled air passed through the laryngeal end of a trans-oral cannula and the other at or near the bifurcation of the trachea. The former thermocouple was an iron-constantan junction and the latter was copper-constantan. The leads from both thermocouples were connected by means of a two-way switch with a Mohl galvanometer having a period of 0.2 seconds. The excursions of the galvanometer were observed directly and recorded manually. By changing the switch after each expiration the inspiratory temperature peaks in the upper and lower portions of the trachea were observed during alternate breaths.

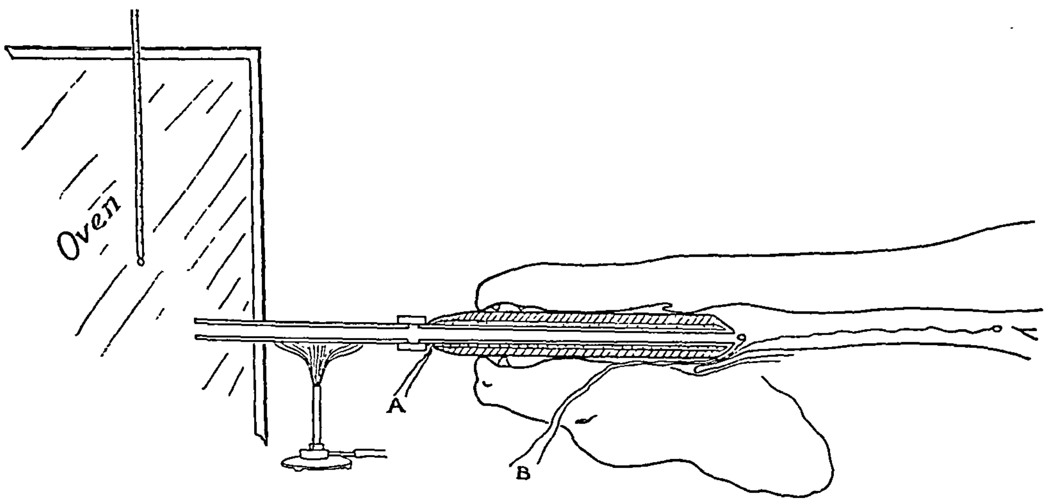
The upper thermocouple was fixed in the center of the laryngeal orifice of the cannula. The lower thermocouple, which was enclosed in a perforated cage to prevent it from coming in contact with tracheal mucous membrane, was inserted to the approximate level of the bifur-

cation by means of a long, slender wire. A certain amount of difficulty was encountered with the deep tracheal thermocouple because of the collection of mucus in the interstices of its protecting cage. It was necessary in some instances to interrupt experiments in order to clean this thermocouple.

Inhalation of Oven-Heated Air

In six experiments the animals breathed room atmosphere that had been heated in an oven (Text-Figs. 1 and 2).

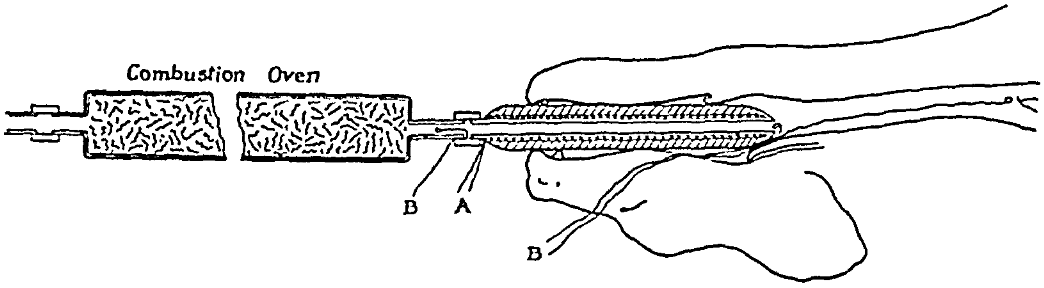
In three experiments (Text-Fig. 1) the temperature of the air as it left the oven was approximately 350° C. and the rate and amplitude of



Text-Fig. 1. Room atmosphere heated to 350° C. Hot air drawn from oven through an insulated trans-oral cannula by the spontaneous inspiratory efforts of the animal. (A) Leads to electrical heating unit in wall of cannula. (B) Leads to thermocouples in laryngeal end of cannula and lower portion of trachea.

respiration depended upon the spontaneous efforts of the animal. A short extension of the trans-oral cannula was inserted through the door of an electrically heated oven which had a capacity of approximately 0.8 cubic meters. A micro burner placed beneath the short metal extension tube tended to prevent cooling of the heated air as it was drawn through it and into the trans-oral cannula. The lining of the cannula was a glass tube having an internal diameter of 6 mm. By passing electricity through a coil of chromel wire which had been wrapped around the inner wall of the cannula, loss of heat during the passage of the air stream through the mouth was reduced. The outer wall of the cannula consisted of a layer of diatomaceous earth covered by lacquered cotton fabric. The internal end of the cannula was well insulated and was shaped to fit the laryngeal orifice snugly enough to prevent any leak of air around it.

In three experiments (Text-Fig. 2) respiration was passive rather than active and the hot air was pumped from a combustion oven into the trans-oral cannula at a rate of 16 strokes per minute, each stroke having a volume of 300 cc. The oven consisted of a bronze cylinder measuring 3 cm. in diameter and 45 cm. in length. To increase its

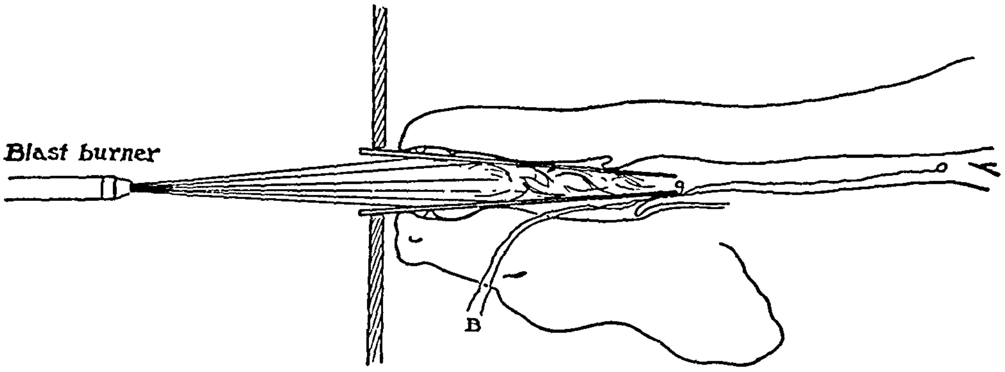


Text-Fig. 2. Inhalation of room atmosphere heated to 500° C. Hot air pumped from combustion oven through insulated trans-oral cannula into trachea. (A) Leads to electrical heating unit in wall of cannula. (B) Leads to thermocouples in outlet of oven, in laryngeal end of cannula and in lower portion of trachea.

heating efficiency the oven was packed with chrome steel shavings. The temperature of the air as it passed out of the oven and into the trans-oral cannula was approximately 500° C.

Inhalation of Flame

In five experiments (Text-Fig. 3) the only atmosphere available to the animal for respiration was the flame and combustion products of a glass blower's blast burner. In these experiments a funnel-shaped trans-oral cannula was used having an internal diameter of 2 cm. at its outer end and of 6 mm. at its laryngeal orifice. The external end of the cannula extended through a hole in a heavy sheet of asbestos board which was placed there to shield the animal's head from the flame.

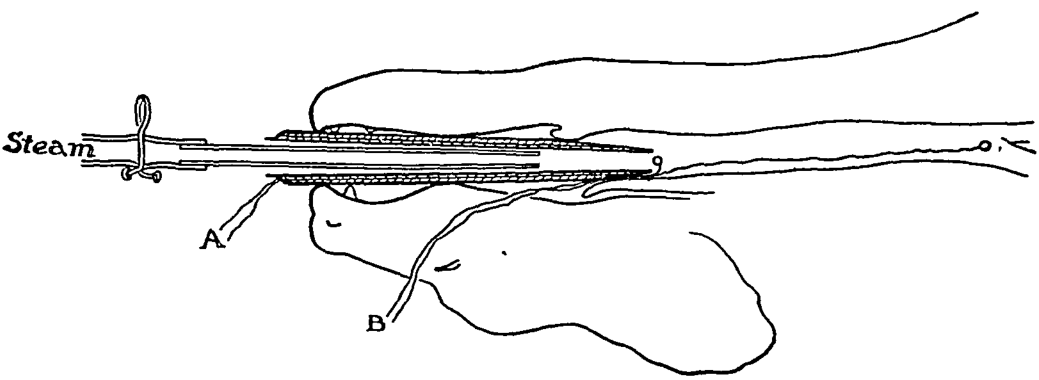


Text-Fig. 3. Inhalation of flame and combustion products from blast burner. Flame entered external end of cannula only during inspiration. (B) Leads to thermocouples in laryngeal end of cannula and in lower portion of trachea.

Approximately 20 cm. in front of the asbestos shield a blast burner was mounted in such a way that its flame could be projected directly into the external orifice of the cannula. The air-fuel mixture was adjusted so that the gas continued to burn throughout the length of the cannula. A shutter consisting of a disk of asbestos was mounted between the burner and the mouth of the cannula so that flame could be excluded from the cannula between inspirations. For the duration of each inspiration the shutter was opened and the flame allowed to enter the cannula.

Inhalation of Steam

In six experiments (Text-Fig. 4) the animals breathed steam which was generated in an 8 liter flask. Two exhaust tubes were passed through the stopper of the flask. The end of the first of these was introduced into the lumen of the trans-oral cannula and was kept closed



Text-Fig. 4. Inhalation of live steam. Steam entered cannula only during inspiration. (A) Leads to electrical heating unit in wall of cannula. (B) Leads to thermocouples in laryngeal end of cannula and in lower portion of trachea.

by means of a spring clamp except during the inspiratory phase of respiration. The wall of the tube was heated by electricity to prevent the condensation of moisture within it. The fit between it and the inside of the cannula was sufficiently loose so that air could be expelled through this compartment during expiration without having to pass back through the tube from which the steam was discharged. The free end of the second exhaust tube from the steam generator was submerged in a column of water through which the steam could bubble when the first tube was clamped. By regulating the height of the column of water in this trap it was possible to develop approximately the same amount of steam pressure within the flask during each interinspiratory interval. The pressure was so regulated that the release for 1 second of the spring clamp on the tube that opened into the cannula led to the discharge of approximately 250 cc. of live steam in some experiments and 400 cc. in others.

Results of Experiments

The number, kinds, and results of experiments performed to determine the effects of heat on the air passages and lungs are summarized in Table I.

Rate of Cooling of Inhaled Air

When superheated air was inhaled, the temperature recorded by both the laryngeal and the deep tracheal thermocouples rose throughout inspiration and fell during expiration. In each situation the highest point in the temperature curve was reached at or near the end of inspiration. As indicated in Table I, relatively dry gases lost most of their heat before reaching the lungs whereas the rate of heat loss from a steam-air mixture during its passage down the trachea was much less rapid. Another difference incident to the inhalation of dry and moist air was the contour of the temperature curves. When hot, dry air was inhaled, the temperature rose to a sharp peak and fell rapidly during expiration. When steam was inhaled, the curve described a plateau rather than a peak. It is likely that this character of the temperature curve during the inhalation of steam was due, in part at least, to the condensation of hot water on the thermocouples. This would tend to dampen their sensitivity and cause them to retain their heat longer than if they had remained dry.

In experiments 1 to 4 the air was inhaled directly from an oven in which the internal temperature was maintained at 350° C. Despite the fact that the airway between oven and larynx was short and that heat loss through its wall was reduced by auxiliary heating devices, the temperature of the air stream fell from 350° C. in the oven to between 159° and 182° C. by the time it reached the larynx.

In experiments 5 to 7 the air was pumped through the trans-oral cannula and into the trachea from a red-hot combustion oven. As the air left this oven its temperature was in excess of 500° C. and yet it fell to between 267° and 327° C. by the time it reached the laryngeal end of the cannula and to 50° C. by the time it reached the bifurcation of the trachea. Since the pump was regulated to discharge 300 cc. with each stroke and since the entire output of each stroke was forced into the trachea, the failure to develop high intrapulmonary temperatures could not be attributed to shallow breathing.

In experiments 8 to 12 the animals breathed the flame and combustion products of a glass blower's blast burner. As already indicated, the burner was so regulated that tongues of blue flame actually reached the laryngeal end of the cannula where air temperatures of between 327° and 550° C. were recorded during each inspiration. Despite this

TABLE I

Summary of Experimental Data

Kind of atmosphere breathed	Animal numbers		Original pre-inspiratory temp. of air (approximate)	No. of breaths	Max. temperature recorded		Recovery period (hrs.)	Site and severity of injury		
					Laryngeal cannula	Lower trachea		Upper trachea	Lower trachea	Lungs
Air from drying oven (See Text-Fig. 1)	1	423	350° C.	46	182° C.	—	19	Mild	None	None
	2	420	350° C.	52	180° C.	—	19	Mild	None	None
	3	391	350° C.	103	159° C.	—	30	Mild	None	None
	4	390	350° C.	106	175° C.	—	Not killed	(Complete clinical recovery—no autopsy)		
Air from combustion oven (See Text-Fig. 2)	5	392	500° C.	60	267° C.	—	4	Mild	None	None
	6	432	500° C.	44	327° C.	50° C.	7	Moderate	None	None
	7	426	500° C.	22	291° C.	—	24	Mild	None	None
Flame from blast burner (See Text-Fig. 3)	8	431	—	17	—	135° C.	7	Moderate	Mild	None
	9	433	—	10	327° C.	51° C.	8	Severe	Moderate	Mild
	10	454	—	16	540° C.	100° C.	11	Severe	Mild	None
	11	455	—	24	550° C.	65° C.	24	Moderate	Mild	None
	12	405	—	14	510° C.	64° C.	Not killed	(Complete clinical recovery—no autopsy)		
Steam from generator (See Text-Fig. 4)	13	456	Over 100° C.	27	106° C.	59° C.	6	Moderate	Mild	None
	14	519	Over 100° C.	18	98° C.	79° C.	7	Severe	Moderate	None
	15	481	Over 100° C.	20	94° C.	53° C.	10	Severe	Severe	Severe
	16	475	Over 100° C.	15	99° C.	94° C.	10	Severe	Severe	Severe
	17	524	Over 100° C.	10	—	90° C.	24	Severe	Severe	Moderate
	18	522	Over 100° C.	12	—	75° C.	48	Severe	Severe	Mild

extraordinary attempt to produce a high intrapulmonic temperature, the highest thermocouple reading at the tracheal bifurcation was 135° C. (experiment 10). In the other four experiments the deep tracheal inspiratory peaks ranged between 51° and 100° C. The reason that the deep tracheal temperature peaks in experiment 10 were so much higher than those obtained in the other experiments with the inhalation of flame was not discovered. Actually no. 10 was the first experiment that was performed in the flame series and so far as is known the same experimental method was followed throughout.

Although the respiration was spontaneous in these experiments, the inhalation of combustion products caused rapid, deep breathing which should have predisposed to the attainment of maximal intrapulmonary temperature levels.

In experiments 13 to 18 a mixture of live steam and air was inhaled. The inspiratory peaks recorded at the laryngeal opening of the cannula ranged between 94° and 106° C. and the temperatures recorded by the deep tracheal thermocouple ranged between 53° and 94° C. Although the temperature recordings in both situations were probably altered by condensation of moisture on the thermocouples, it was obvious that steam had more heat to give up and that it gave it up more slowly than did hot, dry air.

Effects on Animals

As already indicated, it was the purpose of these experiments to expose the air passages and lungs to heat without causing concomitant injury of the skin so that any pathological changes that occurred could be attributed to the inhaled heat independently of any secondary effects that might result from thermal injury of the surface of the body.

Skin. Actually the mildest thermal exposures used in the inhalation experiments were more than sufficient to cause severe injury to the skin. Every animal included in Table I would have sustained extensive cutaneous injury if the skin had been exposed for more than a few minutes to an atmosphere as hot as that which entered the trans-oral cannula. It was observed in pilot experiments on dogs and pigs that air (room atmosphere) temperatures as low as 300° C. produced severe injury of unprotected skin after a 30 seconds' exposure. Mixtures of steam and air at 100° C. destroy epidermis within a few seconds. The destructive effect on a dog's epidemis of a 1 second exposure to steam is shown in Figure 4.

Pharynx and Larynx. Early in the investigation it was found that if animals were to survive an injurious thermal exposure long enough to permit the development of reactive changes in the lower air passages,

it was necessary for the primary impact of the hot air to be below rather than above the larynx. If the air is inhaled in a normal fashion and at a high enough temperature to damage the lower air passages, it is likely to cause death from obstructive edema of the laryngopharynx and larynx (Fig. 5) before sufficient time has elapsed for the development of reactive changes in the deeper portions of the respiratory tract.

In a series of exploratory experiments not included in Table I, the internal end of the trans-oral cannula was modified in such a manner that the first impact of the hot atmosphere during inspiration occurred in the pharynx rather than below the vocal folds of the larynx. In these pilot experiments it was found that several breaths of dry air delivered into the pharynx at a temperature of 300° C. or of steam delivered at 100° C. caused such a severe local edema within a few hours that the animals died of obstructive asphyxia.

The mucosa of the pharynx and larynx of the dog is not uniformly susceptible to the development of edema. The tongue and the laryngeal surface of the epiglottis can sustain severe injury without the occurrence of appreciable swelling. However, a relatively short exposure of the laryngopharynx, the pharyngeal surface of the epiglottis, or of the laryngeal ventricles results in the development of a massive gelatinous mucosal edema which tends to obstruct the airway within a few hours. Severe burning of the lower portion of the larynx or of the trachea may be survived with remarkably little reduction in the patency of the airway.

Thermal Injuries of the Respiratory Tract (Sublaryngeal). It may be seen in Table I that pulmonary injury occurred in none of the 7 animals that breathed oven-heated air, in only 1 of the 5 animals that inhaled flame from a blast burner, and in 4 of the 6 animals that inhaled live steam. In the remaining animals primary injury caused by the inhalation of superheated atmosphere was confined to the upper air passages.

Thermal Tracheitis. Animals 1 to 4 breathed air directly from the 350° C. drying oven and developed a mild catarrhal reaction of the upper respiratory tract. Although the reaction was minimal so far as microscopical appearance was concerned, the copious outpouring of mucus during the first 12 to 18 hours of the recovery period provided evidence of its occurrence. The surface epithelium of the tracheal mucosa remained intact and there was neither edema nor cellular exudation.

Animals (5 to 7), into whose tracheas air had been pumped from a 500° C. combustion oven, developed an acute tracheitis for a distance of 8 to 12 cm. below the larynx. In addition to edema of the subepi-

thelial stroma and evidence of increased secretory activity of mucous glands, animal 6 disclosed a circumscribed patch of mucosal ulceration where the stream of hot air from the orifice of the improperly aligned cannula first struck the posterior wall of the trachea. In none of the animals of this group was there recognizable injury of the lower portion of the trachea, the bronchi, or the lungs.

Animals 8 to 18 breathed flame or live steam and developed tracheitis ranging in intensity from moderate to severe. Although the most severe injuries invariably occurred in the upper portion of the trachea of these animals, catarrhal and in some instances membranous inflammation was prolonged beyond the bifurcation.

Mucosal necrosis with desquamation of surface epithelium occurred in all instances where the blast of hot atmosphere first struck the tracheal wall. In the case of injuries designated as moderately severe the mucosal ulceration was localized. Tracheitis designated as severe was characterized by extensive and, as a rule, completely circumferential ulceration.. In both types of injury the necrotic mucosa desquamated and the denuded surface acquired a thick covering membrane of necrotic epithelium, fibrin, and exudative cells. In the case of severe injuries large casts of necrotic mucosa were expelled by coughing during the first 12 hours of the recovery period.

In injuries designated as moderate the edema was confined to that part of the tracheal wall that lay within the cartilaginous rings. In severe injuries peritracheal edema of varying intensity was encountered. In some instances it was confined to the neck and in others it extended into the mediastinum and even into the peribronchial and perivascular connective tissue of the central portions of the lungs.

Pulmonary Injury. One of the 5 animals that breathed flame and 4 of the 6 animals that breathed a mixture of live steam and air disclosed evidence of primary thermal injury of the lungs in addition to severe injury of the upper respiratory passages.

The portions of the lungs most vulnerable to thermal injury were those centrally located alveolar ducts and communicating alveoli that had the shortest and most direct connections with the large bronchi at the hilum. Atmosphere not hot enough to damage the mucosa of the large bronchi or the alveoli of the peripheral portions of the lungs may still be hot enough to cause edema and hemorrhage of centrally located alveoli. In 3 of the 5 animals in which thermal pneumonitis was recognized, the primary pulmonary injuries were confined to the central portions of the lobes.

In the mildest form of primary thermal injury of the lungs the changes were confined to the terminal portions of centrally located

respiratory bronchioles, their alveolar ducts and the adjacent air sacs. In the early stage of such a lesion the walls of the affected alveoli and alveolar ducts were thickened, in part by edema and in part by hyperemia, and there was an escape of deeply acidophilic serous fluid into the lumen. In more severe injuries of this type, extravasated erythrocytes and fibrin were present in the escaped fluid. Where sufficient heat had reached the lungs to produce such changes, it was usual to find edema of the connective tissue around the larger blood vessels and air passages even though the mucosa of the latter showed little or no evidence of damage. Although there was evidence of capillary injury, as indicated by extravasated erythrocytes, no thrombosis was observed.

An example of what might be characterized as relatively mild central pneumonitis due to inhalation of hot atmosphere is shown in Figure 3. It may be seen that the large bronchi are contracted and show no evidence of mucosal injury. The peripheral pulmonary parenchyma is normal. The central parenchyma is relatively airless due in part to hyperemia and atelectasis and in part to intra-alveolar edema. Occasional small bronchi are obstructed by masses of aspirated mucosal débris. This débris came from the upper portion of the trachea which was extensively ulcerated. A higher magnification of the consolidated central portion of the lung is shown in Figures 11 and 12.

In two animals (15 and 16) which inhaled steam, the pulmonary injury was characterized as severe. In these there was extensive and generalized destruction of the bronchial mucosa. Instead of the usual post-mortem contraction (Fig. 3) the ulcerated bronchi remained widely patent (Fig. 10). Although there was generalized pulmonary edema and hyperemia in these animals, the massive interstitial hemorrhage and interstitial swelling in the hilar regions led to a particularly striking form of central consolidation. Such injuries were observed only in animals that had inhaled steam and the impression was gained that the water-laden atmosphere had sufficient residual heat to cause capillary injury even after it had reached the most outlying portions of the lungs. It was noteworthy that these two animals survived for 10 hours despite the severity of their pulmonary injuries.

Focal patches of atelectasis and emphysema were encountered in animals that had sustained primary pulmonary injury and in those that had not. In some instances these disturbances were obviously due to the aspiration of mucus or mucosal débris from the upper air passages. In others the cause of the obstruction was not discovered. In no instance was it felt that obstruction of the small air passages of this type interfered significantly with pulmonary ventilation.

Bronchopneumonia was observed as early as 8 hours after inhala-

tion of superheated atmosphere and occurred in animals that had, as well as in those that had not, sustained recognizable thermal injury of the lungs. When recognizable thermal injury was confined to the upper air passages it was inferred that pulmonary infection was secondary to the aspiration of mucosal débris.

Bronchopneumonia was observed only in animals that had sustained severe tracheal injury. When the thermal injury was limited to the upper air passages the pneumonia tended to be peripheral rather than central. When primary thermal pulmonary injury had been sustained the pneumonic patches were central and peripheral. Bacteriological examination of the intra-alveolar exudate disclosed a mixed flora with cocci in clumps and chains predominating.

There was no reason to infer that any of the animals whose thermal lesions were designated as mild or moderately severe and limited to the upper air passages would have suffered any significant or prolonged respiratory disturbance. No animal in the entire series of experiments died during the inhalation of hot air. One animal (no 5) died 4 hours after exposure of obstructive laryngeal edema due to defective insulation of the laryngeal end of the trans-oral cannula. None of the animals that inhaled flame died, although it is likely that 9 and 10 would have died of infection if they had not been killed. In both instances there was extensive ulceration of the tracheal mucosa. Animal 12 inhaled 14 breaths of flame and was allowed to survive. This animal was used several months later in another experiment at which time there was neither clinical nor pathological evidence of residual pulmonary injury. It was thought that none of the dogs that breathed steam would have survived, with the possible exception of animal 13. Two of these animals died with massive pulmonary edema within the first 12 hours. Two were obviously suffering from respiratory distress when they were sacrificed after recovery periods of 24 and 48 hours respectively.

Although no animals known to have sustained a thermal pneumonitis were allowed to survive longer than 48 hours after exposure, the pathological changes observed in the lungs within 48 hours permitted certain inferences as to the ultimate fate of these animals if a longer survival period had been allowed. With severe thermal injury of the lungs, the edema incident to widespread injury of the pulmonary capillary bed might be expected to cause death from asphyxia or circulatory failure within 12 hours. Regardless of the mildness of a primary thermal injury of the lung, if the inhaled air was hot enough to cause direct pulmonary damage, the associated tracheal injury might be expected to predispose the animal to widespread infection of the respiratory tract.

That such an infection would be likely to lead to the formation of pulmonary abscesses was indicated by its observed severity in those portions of the lung that were obstructed by necrotic masses of aspirated mucosal débris.

Discussion

As indicated in the introductory paragraphs, this investigation was undertaken to determine the pathological characteristics of pulmonary lesions caused by the inhalation of hot atmosphere. Although the relative immunity of the lungs to thermal injury might have been anticipated if due consideration had been given to the thermodynamics of the situation, these factors were not given adequate consideration until after the experiments had been performed.

Why is it that air hot enough to burn the skin of the face can be inhaled without causing damage to the trachea or lungs? Surely the mucosa of the air passages is more delicate and should therefore be more vulnerable to thermal injury than the epidermis. The explanation of the unanticipated results of this investigation lies in the fact that the quantity of heat that can be stored in the volume of gas that constitutes a breath is remarkably small. At any given air temperature the number of calories that can be transferred to the respiratory tract incident to the inhalation of a breath of hot air is limited by the volume of that breath, whereas in the case of the surface of the body where convection currents are capable of bringing a practically unlimited volume of hot air in contact with the skin, an infinitely greater caloric transfer can occur for each unit of surface area exposed.

An illuminating illustration of this difference is provided by calculating the heat transfer that could occur in the respiratory tract if air were inhaled at 142° C. Let it be assumed that the amount inhaled with each breath would be sufficient to increase the pulmonary volume by 500 cc., that the air was dry when inhaled and saturated with moisture when exhaled and that it was cooled to body temperature by the time it was exhaled. The cooling of one such breath from 142° to 38° C. would release approximately 13 calories of heat energy in the body. Theoretically this amount of heat would be sufficient to raise the temperature of 1 gm. of tissue by approximately 13° C. Actually the liberation of this amount of heat would produce no net change in the temperature of the respiratory tract because it would be offset by a loss of 13 calories due to the evaporation of the 23 mg. of water that would be required to saturate that amount of dry air. This is not to imply that the inhalation of air heated to 142° C. would be necessarily harmless. Desiccation or even burning might occur near the portal of entry even though no net change in the temperature of the respiratory tract occurred.

Although there are probably no nonexperimental circumstances in which thermal injury of the respiratory tract can be sustained without the occurrence of concomitant and more severe burning of the exposed surface of the body, there is great variation in the potentiality of different kinds of atmosphere for the production of thermal injury. This variation does not depend so much on differences in heat capacity of the inhaled gases (or material suspended in them)as it does on differences in their capability for heat output through condensation of moisture as the gases are cooled within the body.

Thus, to use the same type of calculation for moist air as was used for dry air, let it be assumed that a mixture of equal parts of steam and air was inhaled at a temperature of 125° C. Such an atmosphere will cause severe burning of the skin within a second. If an amount of such a mixture sufficient to increase the lung volume by 500 cc. were inhaled and if it were cooled to 38° C. before being exhaled, approximately 300 mg. of water would be condensed in the respiratory tract. The heat energy liberated, incident to the condensation of this amount of water, would be approximately 175 calories. In addition to this, approximately 12 calories would be liberated incident to the cooling of the gases and 26 calories incident to the cooling of the condensed water, bringing the total transfer of heat to the respiratory tract in excess of 200 calories. This probably represents a greater transfer of heat than would occur incident to the inhalation of dry air at conflagration temperatures.

Another matter for consideration is the extent to which the results of these experiments can be applied to man. Is it fair to assume that man and dog would be similarly affected by the inhalation of superheated atmosphere under nonexperimental conditions? If hot air were inhaled under ordinary conditions, it seems likely that the relatively long trachea of the dog would make him less vulnerable to thermal pulmonary injury than man. The air would have to travel farther in the dog and would have a better opportunity to give up its heat before reaching the lungs. It seems highly probable, however, that the elaborate precautions that were taken in the animal experiments to prevent loss of heat from the inhaled air as it was conducted to the larynx would more than compensate for the shortness of the human trachea.

Conclusions

1. In experiments on dogs it was observed that only when the original temperature of the air was high enough to produce almost instantaneous burning of the skin and upper respiratory mucosa was

there sufficient residual heat in the air reaching the lungs to cause pulmonary injury.

2. Within the range covered by this investigation no type of thermal pulmonary exposure was encountered which was immediately incompatible with life. A thermal exposure sufficient to injure the lungs was more than enough to cause a rapidly fatal obstructive edema of the glottis. Only when the larynx was protected against heat did animals survive long enough to develop the characteristic lesions of thermal pneumonitis.

3. At any given temperature moist air has more heat to give up than does an equal volume of dry air and is accordingly more likely to cause thermal injury of the respiratory tract.

4. Inhalation of dry or moist hot air may destroy the upper tracheal mucosa without causing primary thermal injury of the lungs. In the dog a severe thermal tracheitis predisposes to the development of bronchopneumonia.

5. The most vulnerable portion of the lung to thermal injury is the central parenchyma where the respiratory bronchioles and alveoli have the shortest and most direct connection with the primary bronchi.

6. In instances of mild thermal injury of the lungs the centrally located alveoli were the seat of hemorrhagic edema even though there had been insufficient heat to cause recognizable injury of the bronchial mucosa or of the more peripherally located air sacs.

7. In instances of severe thermal injury of the lungs there was extensive destruction of bronchial mucosa, dilatation of large and small bronchi, hemorrhagic edema of the peribronchial connective tissue and generalized hyperemia and hemorrhagic edema of the peripheral as well as of the central pulmonary parenchyma.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

Plate 56

Fig. 1. Thermal laryngitis and tracheitis without pulmonary injury. Photograph of respiratory tract of dog 24 hours after inhalation of flame. Sufficient heat had been conducted through the wall of the cannula to cause a mild degree of laryngeal edema which may be recognized by the bilateral olive-shaped mucosal protrusions from the ventricular recesses. There was extensive destruction of the mucosa of the upper trachea, diminishing rapidly to a mild catarrhal inflammation in the lower third. No abnormality of the bronchi or lungs of this animal was recognized.

Fig. 2. Thermal tracheitis and pneumonitis. Photograph of respiratory tract of dog 10 hours after inhalation of steam, showing severe tracheobronchitis with dilatation of the bronchi. There is a central hemorrhagic pneumonitis with generalized pulmonary edema and hyperemia.

Fig. 3. Primary thermal pneumonitis. Photomicrograph of lower lobe of dog's lung 24 hours after inhalation of steam. Although there was severe tracheitis, the primary and secondary bronchi showed remarkably little change. Evidence of pulmonary injury was confined largely to the central portions of lobes and consisted of hyperemia, edema and partial atelectasis. $\times$ 9.

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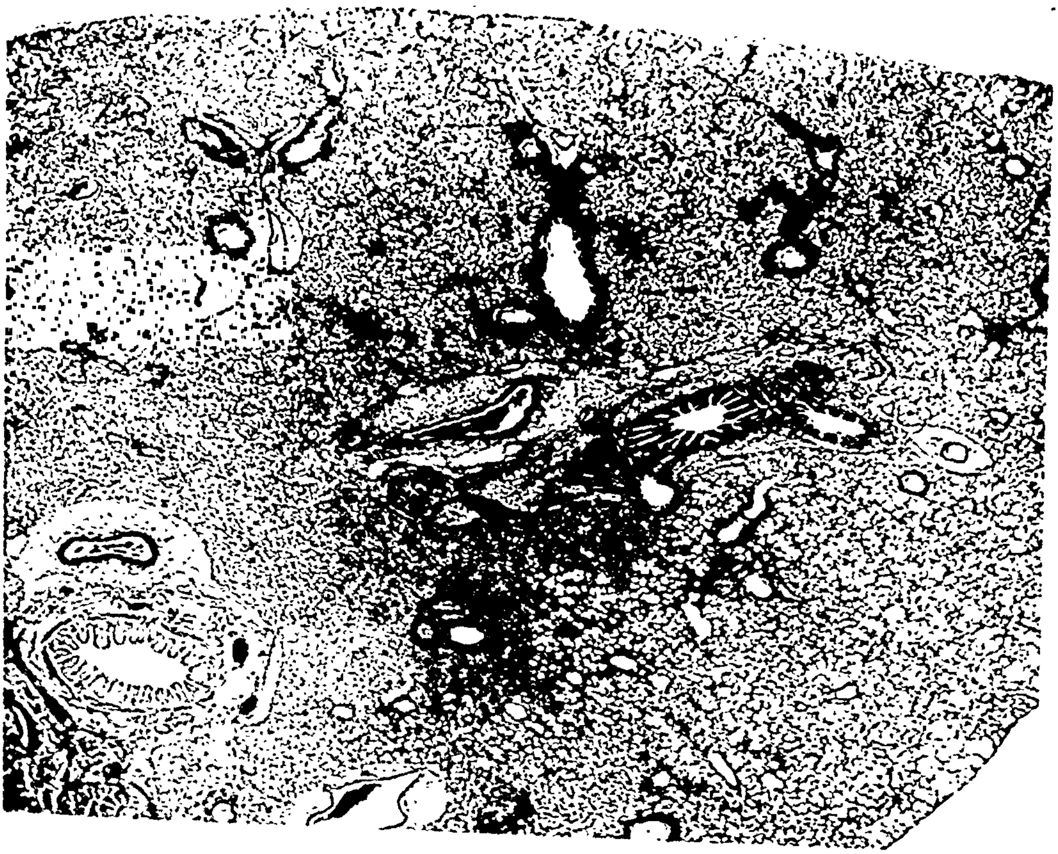

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PLATE 57

FIG. 4. Steam burn of dog's skin. Photomicrograph showing complete necrosis and beginning separation of epidermis from corium 24 hours after a 1 second exposure to live steam. × 80.

FIG. 5. Obstructive edema of glottis due to inhalation of hot air. Photograph of pharynx and glottis of a dog 4 hours after inhalation of hot (500°C.) air. In this experiment the first impact of the hot air was above rather than below the glottis.

FIG. 6. Thermal tracheitis. Photomicrograph of the mucosa of upper portion of trachea 24 hours after inhalation of flame from blast burner. The necrotic superficial epithelium has desquamated and the underlying stroma is desiccated. Beneath the zone of desiccation there is severe edema. The appearance of the lower tracheal mucosa of this animal is shown in Figure 8. × 55.

FIG. 7. Cast of tracheal mucosa expelled approximately 8 hours after inhalation of steam. × 160.

FIG. 8. Catarrhal tracheitis. Photomicrograph showing stromal edema and distended goblet cells in mucosa of lower trachea 24 hours after inhalation of flame. The appearance of the mucosa of the upper trachea of this animal is shown in Figure 6. × 305.

FIG. 9. Normal lung 24 hours after inhalation of flame. Photomicrograph showing wall of large bronchus and adjacent alveoli. Figures 6 and 8 show condition of trachea of the same animal.

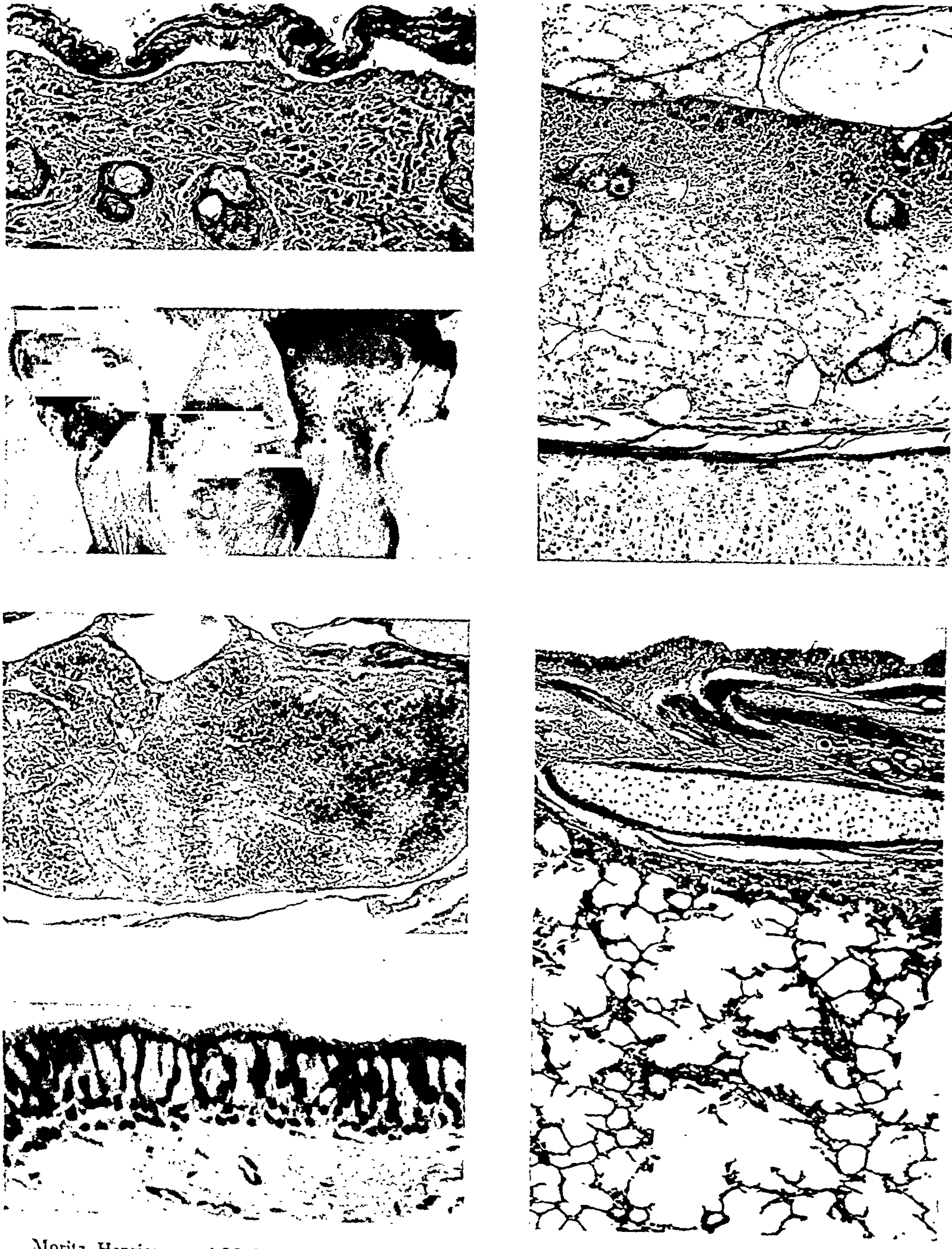

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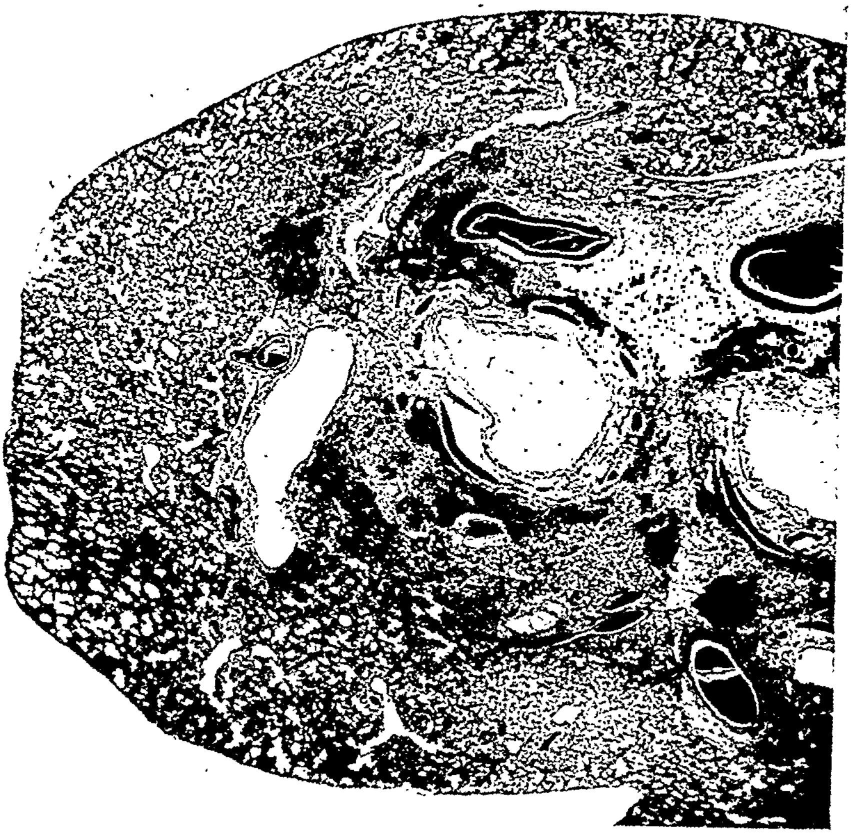
PLATE 58

FIG. 10. Severe thermal pneumonitis. Photomicrograph of section near hilum of lung showing hemorrhagic edema of central interstitial tissue, dilatation of ulcerated bronchi, and generalized edema and hyperemia of alveoli 10 hours after inhalation of steam. The macroscopical appearance of the lungs of this animal was similar to that shown in Figure 2. × 7.

FIG. 11. Thermal pneumonitis. Photomicrograph showing edema of terminal bronchiole in central portion of lung 24 hours after inhalation of steam. The partially collapsed adjacent alveoli are hyperemic and contain many extravasated erythrocytes. × 120.

FIG. 12. Thermal pneumonitis. Photomicrograph showing terminal bronchiole partially obstructed by aspirated mucosal débris and exudate 24 hours after inhalation of steam. There is partial atelectasis of the surrounding alveoli, associated with hyperemia and edema. × 65.



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Effects of Inhaled Heat

A MORPHOLOGICAL STUDY FOLLOWING THE INTRAVENOUS ADMINISTRATION OF GELATIN SOLUTIONS TO DOGS *

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A group of studies has been carried out recently in an effort to determine the suitability of gelatin solutions as nonhematogenous blood substitutes. Ivy, Greengard, Stein, Grodins and Dutton,[1] and also Lawson and Rehm [2] have studied the effectiveness of gelatin solutions in the treatment of hemorrhagic shock in dogs. The use of such solutions in preventing shock induced by trauma to leg muscles and by tourniquets was reported by Kleinberg and his collaborators.[3] The permeability of damaged capillaries to gelatin has been studied by Little and Wells.[4] The effect of intravenous gelatin upon plasma retention, the rate of urinary excretion and the total circulating red cell volume in normal dogs and in dogs with diminished plasma volumes were reported by Little and Dameron.[5,6] *In vivo* conglutination of erythrocytes following the intravenous administration of gelatin has been studied by Miller and Little.[7]

Before a blood substitute may be accepted as a therapeutic agent, it must be demonstrated conclusively that there are no permanent pathological changes following the repeated administration of the substance. Hueper [8] has reported the occurrence of degenerative vascular disease in dogs following the intravenous administration of gelatin solutions of high concentration. In his experiments, initial injections of 2 cc. of a 10 per cent solution of gelatin were given daily, and both the concentration and dose were gradually increased until finally 120 cc. of a 25 per cent solution were being administered. The lesions found in these animals were primarily of a sclerotic type and were noted in the aorta and in the coronary and renal arteries. They consisted principally of a marked fibrous thickening of the intima with medial necrosis and hyalinization. Hueper concluded that the lesions present in these animals were directly attributable to parenterally introduced solutions of gelatin, although he noted further that the lesions were not so striking as those found following the injection of other macromolecular substances.

In view of the fact that gelatin solutions of extremely high concentration were used in Hueper's [8] experiments, it was deemed advisable to make a detailed pathological study of a number of dogs following the repeated administration of gelatin solutions in concentrations more closely approaching those likely to be used clinically.

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Material and Methods

Eight mongrel dogs were given 12 daily intravenous injections, each injection being equivalent to 1 per cent of their body weight, of either 6 per cent or 4 per cent gelatin in aqueous 0.9 per cent sodium chloride solution (Table I). One dog (no. 5) was given only 2 injections of the 6 per cent gelatin solution. The gelatin was prepared by hydrolysis of beef bone and had an iso-electric point near pH 5. At varying intervals following the last injection, the animals were killed with chloroform or by the intravenous injection of sodium pentobarbital.

Autopsies were performed immediately and sections were removed from all organs, special care being taken to obtain adequate material from the vascular, hematopoietic and nervous systems. Three sections were taken from each of the anatomical divisions of the aorta, since this organ has been especially incriminated in previous experimental work. The material removed for microscopical study was fixed in Zenker's fluid-formaldehyde solution and in a 4 per cent solution of neutral formaldehyde. All tissues were cut at 6 μ and stained routinely with hematoxylin and eosin, Mallory's connective tissue stain, and Masson's trichrome stain, and by Weigert's method for elastic fibers.

Table I

Summary of Daily Injections of Gelatin Solution with Each Administration Equivalent to 1 Per Cent of the Body Weight

Dog no.	Weight	Days receiving 6% gelatin	Days receiving 4% gelatin	Days intervening between last injection and sacrificing
1	17 kg.	12	—	2
2	15 kg.	12	—	19
3	10 kg.	12	—	10
4	15.5 kg.	12	—	24
5	6.5 kg.	2	—	1
6	14.5 kg.	8	4	3
7	18.0 kg.	8	4	7
8	21.5 kg.	8	4	17
9	22.0 kg.	8	4	25

Results

Except in dog 1, the pathological findings were confined to the aorta, the kidneys and the coronary arteries. For purposes of description, the lesions may conveniently be grouped according to the structures affected.

Aorta. The most common abnormal findings in the aorta were nodular thickenings in the intima of the abdominal portion of the vessel (dogs 2, 6, 7, 8 and 9). These nodules were composed of deli-

cate elastic lamellae and fibrous tissue. There was thickening of the intima in the immediate vicinity of the nodules and occasionally in situations quite remote from them.

Degenerative changes in the elastic tissue of the media were noted in all of the dogs, although the degree of degeneration varied greatly and in many instances was minimal. The areas left vacant by the elastic tissue were filled in with smooth muscle and connective tissue cells. This process was practically confined to the ascending aorta and arch of the vessel and increased in severity as the aortic valve was approached. Dogs 1, 4, 7 and 8 showed, in addition to these changes, degeneration of elastic tissue and collagen which resulted in cyst formation. Also, in dogs 5 and 7 a scar was seen in the ascending aorta and calcium deposits were present in the immediate vicinity of the valve.

Aortic bacterial endocarditis associated with *Dirofilaria immitis* infestation was seen in dog 1. The liver and spleen were enlarged and clear fluid was present in the serous sacs.

Coronary Arteries. In only one dog (no. 7) could a lesion be demonstrated in the coronary arteries. This abnormal finding consisted of a localized thickening of the subendothelial connective tissue of the intima in one of the larger coronary radicles, with some encroachment upon the lumen of the vessel.

Kidneys. Five of the dogs (nos. 3, 4, 5, 6 and 8) showed chronic interstitial nephritis. In three animals (nos. 2, 7 and 9) no pathological changes could be demonstrated in the kidneys. Dog 1 presented a typical focal nephritis, identical with that seen in the kidneys of persons suffering from bacterial endocarditis.

Discussion

When the data from these experiments are examined, it is readily seen that there is no correlation between the pathological lesions exhibited by these animals and the administration of gelatin. The lesions are in general those of vascular degeneration and repair. In most instances sufficient time did not elapse between the beginning of the experiment and its termination to permit the development of lesions of the type found. Furthermore, if the lesions were produced by gelatin there should be some correlation between the duration of the experiment and the degenerative and reparative processes.

Bacterial endocarditis associated with elastic tissue and collagenous degeneration of the aorta and cyst formation was present in dog 1, which was sacrificed 14 days after the beginning of the experiment. Elastic tissue destruction and replacement fibrosis were seen in dog 5, although this animal was killed 3 days following the initial injection

of gelatin. Dogs 2 and 3 presented neither cystic nor sclerotic lesions, although 31 and 22 days respectively had elapsed since the beginning of the experiment.

Intimal thickening was noted in five animals which were sacrificed within 15 days (dog 6) to 37 days (dog 9) after the initial gelatin injection.

Spontaneous arteriosclerosis in the dog has been described, and it even has been suggested that only animals below the age of 2 years should be employed in experimental work involving a consideration of the vascular system.[9]

Chronic nephritis is a common finding in dogs and Bloom [10] has recently demonstrated that in the great majority of instances it is interstitial in type. In 200 animals dying of various causes, he found that 108 showed focal interstitial nephritis.

Bacterial endocarditis is a rare disease in dogs. However, it has been seen in association with *Dirofilaria immitis* infestation,[11] although most animals which are infected with this parasite show no abnormalities of the heart valves. Faust [12] stated that he has never observed the condition.

Spontaneous medionecrosis with or without cyst formation and smooth muscle replacement has not been described in the dog, although Rottino [13] has demonstrated degenerative lesions with similar smooth muscle and connective tissue changes in 95 of 210 unselected human aortas. Further, it is significant that the medionecrosis in human aortas described by Erdheim [14] and Moritz,[15] and of frequent occurrence in our work, has not been described in the dog.

Finally, it is interesting to note that during the course of our investigation Koop and his associates [16] concluded that there were no permanent pathological changes occurring in normal dogs as a result of repeated infusions of gelatin over long periods of time.

Summary and Conclusions

Nine mongrel dogs were given daily injections of gelatin solutions in concentrations most likely to be used clinically. These animals were sacrificed after varying periods of time, and degenerative and reparative changes, confined for the most part to the aorta, were found in the vascular system. Inflammatory lesions present in the kidneys of more than half of the dogs were similar to the interstitial nephritis commonly seen in these animals and recently emphasized by Bloom.[10]

If the degenerative, reparative and inflammatory changes present in these animals had resulted from the parenteral administration of gela-

tin solutions, one would expect to find some correlation between these processes and the duration of the experiment. No such correlation could be noted.

It is apparent from recent work that many investigators have not taken into account spontaneous aortic disease in the dog in evaluating their experimental results. In an attempt to clarify the problem further, it was deemed advisable to sacrifice for comparison a series of supposedly healthy mongrel dogs and to carry out carefully executed anatomical studies. The lesions seen in the control animals did not differ fundamentally from those seen in the ones which had received injections of gelatin. Since spontaneous vascular disease in the dog does not appear to be generally recognized, it was thought that the vascular lesions in both groups of animals were of sufficient interest to warrant an additional and more detailed report.[17]

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CHANGES IN THE BLOOD VESSELS OF APPARENTLY HEALTHY MONGREL DOGS *

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As a result of anatomical studies made in a group of dogs which had received gelatin solutions parenterally, it was concluded that there were no permanent pathological lesions in these animals which could be directly attributed to the gelatin.[1] Degenerative and reparative processes in the vascular system were of frequent occurrence, but since there was no correlation between the duration of the experiment and the type of lesion found, it was concluded that the changes were spontaneous and had no relation to the experimental procedure employed. This view was strengthened further by the fact that the vascular changes in the experimental animals were identical morphologically with those which have been described in a large percentage of human aortas removed from persons dying of various causes.

A review of the literature reveals that many investigators have not appreciated fully the existence of spontaneous vascular disease in the dog. It is the purpose of this paper to point out certain pathological changes occurring in this animal which have recently received considerable attention in human material but have not been described in the dog.

MATERIAL AND METHODS

Eleven mongrel dogs were chosen at random from the City Dog Pound and were killed immediately by shooting or by the intravenous administration of sodium pentobarbital. Five puppies, approximately 6 months old, and 3 additional ones, 10 days old, were sacrificed by the same methods. It was thought permissible to include 9 additional dogs utilized in another experiment, since it was concluded from that study that there were no abnormal alterations in the tissues which could be attributed to the experimental procedure.[1]

Autopsies were performed immediately and numerous blocks were removed from all organs, special care being taken to include several blocks from each of the anatomical divisions of the aorta, the heart, and the kidneys. The tissue was fixed in Zenker's fluid-formaldehyde solution and also in a 4 per cent solution of neutral formaldehyde. All material was sectioned at 6 μ and stained routinely with hematoxylin and eosin, Mallory's connective tissue stain, and Masson's trichrome stain, and by Weigert's method for elastic fibers.

* Received for publication, April 20, 1944.

Results

The lesions found in these animals were confined for the most part to the vascular system and kidneys, and consisted of degenerative and reparative changes in the former and inflammatory lesions in the latter. For purposes of description the anatomical changes have been classified according to the organs involved.

Aortic Lesions

The aortic lesions were of four principal types:

1. *Focal loss of elastic tissue with grouping of smooth muscle cells* was most marked in the ascending aorta and arch of the vessel. It was displayed in varying degrees by all animals, but was much less prominent in the 10-day-old puppies. The lesions were characterized by thinning of the elastic lamellae and, in many instances, by interruption of their continuity. Some areas were practically devoid of elastic tissue (Fig. 1). The lamellae adjacent to these areas were compact and the interlamellar spaces were greatly narrowed. The areas devoid of elastic tissue were filled in by groups of spindle-shaped cells possessing oval or spherical nuclei which were surrounded by an abundant cytoplasm (Figs. 2 and 3). In some instances the nuclei were hyperchromatic, while in others the chromatin was granular and tended to accumulate at one pole of the cell. In tissues prepared by Masson's trichrome method, the cytoplasm of the spindle cells stained a deep red, and blue-staining collagenous fibers radiated in all directions among the cells. In some instances bundles of collagenous connective tissue fibers were seen, and occasionally they formed minute scars (Fig. 4). The spindle-shaped cells appeared for the most part to be smooth muscle cells, but a varying number of fibroblasts were scattered throughout the areas. Many of the cells were arranged in a circular manner, but not infrequently they pursued an oblique or longitudinal direction (Fig. 3).

2. *Medial necrosis with cyst formation* was pronounced in only 2 dogs, but was seen in varying degrees in 8 animals. It did not occur in either group of puppies. The lesions were most marked in the ascending aorta and arch of the vessel. In the focal areas of degeneration the tissues normally composing the media had disappeared and had been replaced by a basophilic substance which not infrequently contained cells in various stages of degeneration (Fig. 5). In certain instances a delicate fibrillar material could be demonstrated within the cysts, and occasionally intracystic collagenous deposits were noted.

3. *Intimal thickening with splitting and reduplication of the internal elastic lamina* was present in 14 dogs and was confined to the abdominal portion of the aorta. In 7 animals nodular formations projected into the lumen of the vessel (Fig. 6), while in the remainder only a localized

thickening was noted (Fig. 7). Smooth muscle and connective tissue cells and varying amounts of collagen were present in addition to the elastic tissue. Changes of this type could be demonstrated in all groups of animals, including the 10-day-old puppies (Fig. 8). Five dogs showed localized thickening of the intima with marked fibrosis and hyalinization. In 3 animals sections prepared from the aorta near its bifurcation showed thickening and disruption of the internal elastic lamina with fibrous thickening of the intima and nodule formation (Fig. 9).

In every animal certain sections revealed a separation of the lamellae of the intima resulting in the formation of irregular cystic spaces (Fig. 10). This phenomenon was best seen in the ascending aorta and arch of the vessel.

4. *Localized areas of hyalinized collagenous tissue with calcification* were present in 5 dogs and were confined to the ascending aorta (Figs. 11 and 12). They were seen in the intima and media of the vessel, and in no instance was the process extensive. This finding was not seen in any of the puppies.

Sclerosis of the Coronary Artery

In only one animal was there any evidence whatsoever of sclerosis of the coronary artery. In this instance the abnormality was limited to a minimal fibrous tissue thickening of the intima of one of the larger coronary radicles.

Bacterial Endocarditis

One dog presented lesions identical with those ordinarily seen in cases of bacterial endocarditis in man. The heart and liver were enlarged and weighed 250 and 960 gm. respectively. Free fluid was present in all of the serous cavities and there was an increase in density in the more dependent portions of the lungs.

When the heart was opened, large numbers of *Dirofilaria immitis* were found in the right side of the organ. Numerous friable vegetations were present on the aortic valve (Fig. 13), and considerable hyalinized fibrous tissue had been deposited within it. The microscopical picture was identical with that seen in the human disease, and Gram-positive cocci were present in large numbers within the vegetations. The liver and lungs of this animal were congested and there were areas of focal nephritis (Fig. 14).

Chronic Interstitial Nephritis

Inflammatory kidney disease was present in 11 dogs and was characterized by plasmacytic and lymphocytic infiltration. The lesions were confined for the most part to the interstitial tissue, and large groups of

inflammatory cells surrounded the tubules, blood vessels and glomeruli (Figs. 15 and 16). A generalized increase in fibrous tissue was noted in most instances, and in some instances small areas of renal parenchyma were destroyed and replaced by fibrous tissue. The arterioles in these areas were frequently sclerotic. Occasionally small amounts of calcium salts had been deposited in the degenerated areas (Fig. 17). Minimal hyaline deposits in the intima of the larger renal vessels were quite common and occurred in kidneys which were otherwise normal with the same frequency as in those which were the seat of interstitial nephritis. These changes were confined to adult dogs.

Discussion

A review of the literature reveals that canine pathology has, in general, been a greatly neglected subject. In recent years, however, several attempts have been made to study arteriosclerosis in the dog. Most workers in this field restricted the use of the term arteriosclerosis to those instances in which there were localized areas of degeneration in the intima associated with fatty deposits and plaque formation. Other authors employed the term in the broad sense "as a collective and descriptive term covering the changes without active inflammation, that affect the smoothness, thickness, uniformity, homogeneity of tissue, elasticity of the vessel wall and the maintenance of a normal lumen."[2]

Köllisch[3] examined the aortas from 100 dogs and, employing the term to include all degenerative vascular changes, stated that he found arteriosclerosis in 23 of them.

Strauch[4] studied the aortas from a group of dogs ranging from 1 to 5 years of age and also from a group of older dogs. Restricting his use of the term to those instances in which intimal degeneration, with fatty deposits and plaque formation, was demonstrable, he was able to demonstrate true arteriosclerosis in only one animal in the younger group. In the older group he found arteriosclerosis to be a fairly common lesion. This author also found in young and in old dogs a widespread degeneration of the outer layers of the media, associated with scar formation and some thickening of the intima. These changes were confined to the abdominal portion of the vessel. "Stripes" or "ridges" were present in many of the vessels, and Strauch attributed them to the state of contraction of the media. These changes were present in the aorta of nearly every animal examined, and were most marked near the origin of the vessel.

In a recent study of human aortas removed routinely at autopsy, Rottino[5] found medial degeneration in approximately one-half of them.

The majority of the vessels examined showed only simple muscle loss, but elastic tissue degeneration and cyst formation were present in some instances. These changes have not been described previously in the dog, but a large number of our animals presented lesions morphologically identical with those seen in the human material studied by Rottino. The most common lesions consisted of focal loss of elastic tissue and grouping of smooth muscle cells, with or without associated collagenous deposits. In many instances the smooth muscle cells appeared to be actually increased in number, although it was difficult to ascertain this with certainty. Medial necrosis with cyst formation, identical with that described for the human aorta,[6-8] was seen. Hyalinization of the intima and media, associated with calcium deposits, was found in 5 of our animals. It has been pointed out by Nieberle[9] that this sometimes occurs in the first part of the aorta of dogs.

Localized areas of intimal thickening characterized by splitting and reduplication of the internal elastic lamella were present in a large number of animals and were seen even in puppies only 10 days of age. It has been noted previously[2] that this is a common finding in lower animals. Folding of the intima with separation of the elastic lamella was seen in every animal and was most marked in the thoracic portion of the vessel. This, however, is probably due to post-mortem contraction of the media.

It can readily be seen that spontaneous aortic disease is very common and occurs in animals of all ages. However, medial necrosis associated with cyst formation and collagenous deposit is seen only in adult dogs. Pronounced lesions in vessels other than the aorta are extremely rare, and in only one instance was intimal thickening seen in the smaller vessels. Hyaline deposits were frequent in the intima of the larger vessels in the kidney, but there was no thickening of the intima or encroachment upon the lumen of the vessel.

Chronic interstitial nephritis was common in these animals, and the changes were morphologically identical with those recently described by Bloom.[10] Bloom has pointed out that other types of nephritis, such as pyelonephritis and glomerulonephritis, are extremely rare in the dog.

One of our animals showed bacterial endocarditis which was morphologically identical in every way with that seen in man. The accidental finding of bacterial endocarditis in dogs is extremely rare. One cannot say with certainty what part infestation of the blood stream with *Dirofilaria immitis* played in the development of this disease. A careful search of the literature revealed no reference to bacterial endocarditis associated with *Dirofilaria immitis* infestation in dogs. Faust[11] stated

that he had never observed the two conditions in the same animal. However, in a personal communication Brown[12] stated that he had observed animals similar to the one described here.

Summary

In 28 mongrel dogs sacrificed for purposes of anatomical study, there was found to be a high incidence of degenerative changes in the aorta and of inflammatory lesions in the kidney. The aortic lesions consisted of focal loss of elastic tissue with grouping of smooth muscle cells, medial necrosis with cyst formation, intimal thickening with splitting and reduplication of the internal elastic lamina, and localized areas of hyalinized collagenous connective tissue with calcification. Atheromatous plaques were not present in this material, although localized intimal thickenings were of common occurrence and were seen in all groups of animals, including very young puppies. Hyaline deposits were frequently present in the intima of the larger branches of the renal artery, and in one instance intimal thickening was noted in a coronary vessel.

Chronic interstitial nephritis was present in more than one-half of the fully grown animals, and one dog suffered from bacterial endocarditis associated with *Dirofilaria immitis* infestation.

Unless the investigator familiarizes himself with the vascular changes which commonly occur in the dog, he is apt to attribute such changes erroneously to the experimental procedure employed.

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[*Illustrations follow*]

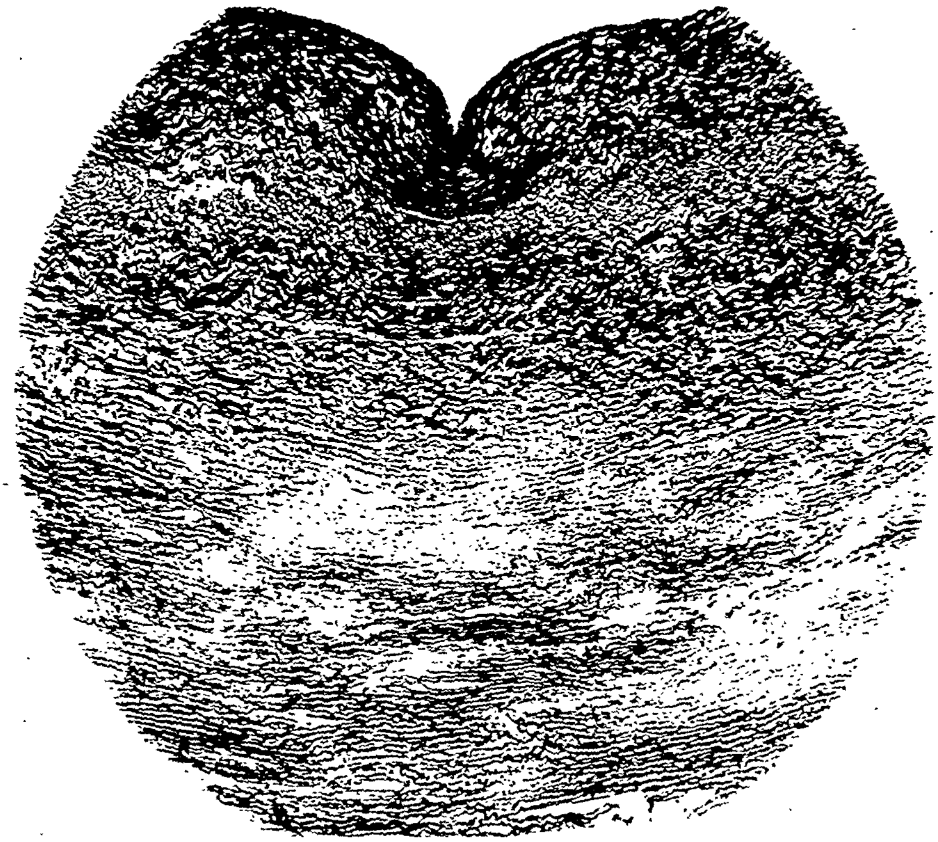
DESCRIPTION OF PLATES

PLATE 59

FIG. 1. Focal loss of elastic tissue. Weigert's elastic tissue stain. $\times$ 110.

FIG. 2. Focal loss of elastic tissue with grouping of smooth muscle cells. Masson's trichrome stain. $\times$ 110.

FIG. 3. An area of grouped muscle cells from the same aorta as that used for Figure 2. $\times$ 250.



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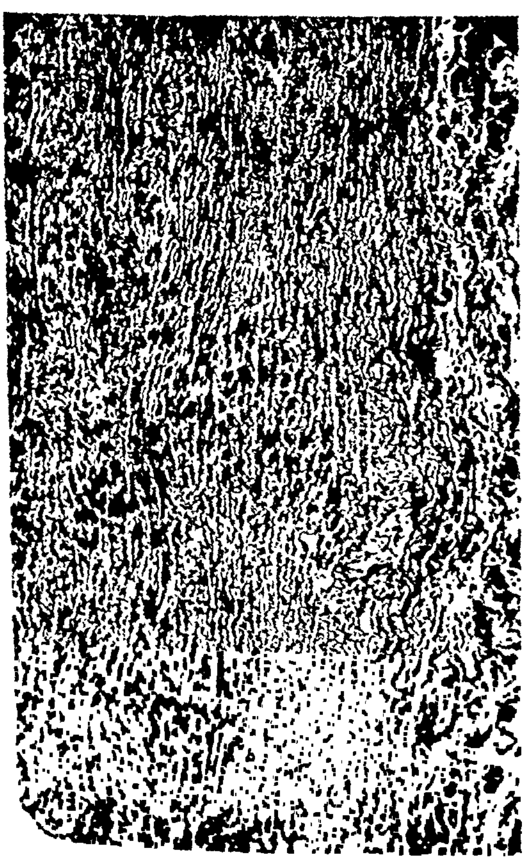

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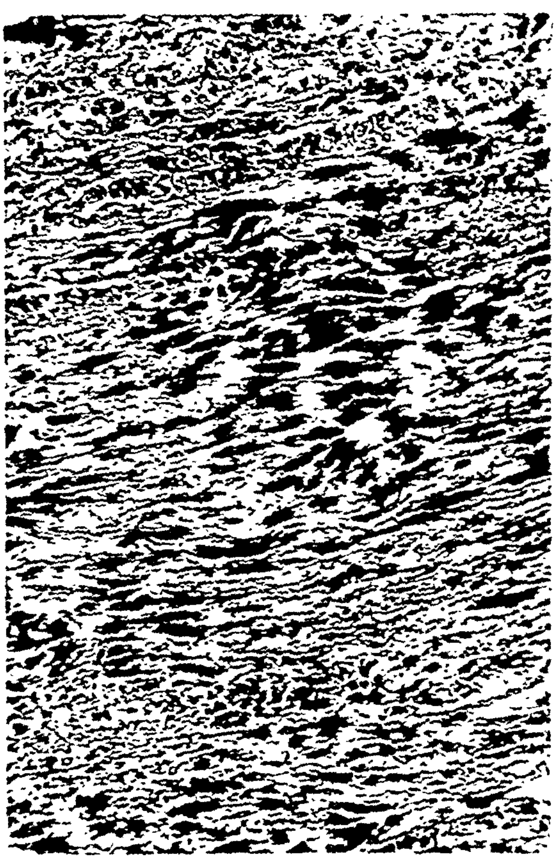

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Morehead and Little Blood Vessels of Apparently Healthy Dogs

PLATE 60

FIG. 4. Collagenous deposits in the media. Masson's trichrome stain. $\times$ 250.

FIG. 5. Medial necrosis with cyst formation. $\times$ 110.

FIG. 6. Splitting and reduplication of the internal elastic lamina with nodule formation. Weigert's elastic tissue stain. $\times$ 60.

FIG. 7. Thickening of the intima with splitting and reduplication of the internal elastic lamina but without nodule formation. Weigert's elastic tissue stain. $\times$ 60.

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Morehead and Little

Blood Vessels of Apparently Healthy Dogs

Plate 61

Fig. 8. The same process as shown in Figure 6, occurring in a 10-day-old puppy. Weigert's elastic tissue stain. × 60.

Fig. 9. Thickening and disruption of the internal elastic lamina with fibrous thickening of the intima and nodule formation. This section was taken from the aorta near its bifurcation. Weigert's elastic tissue stain. × 250.

Fig. 10. Separation of the lamellae of the intima resulting in the formation of irregular cystic spaces. Masson's trichrome stain. × 110.

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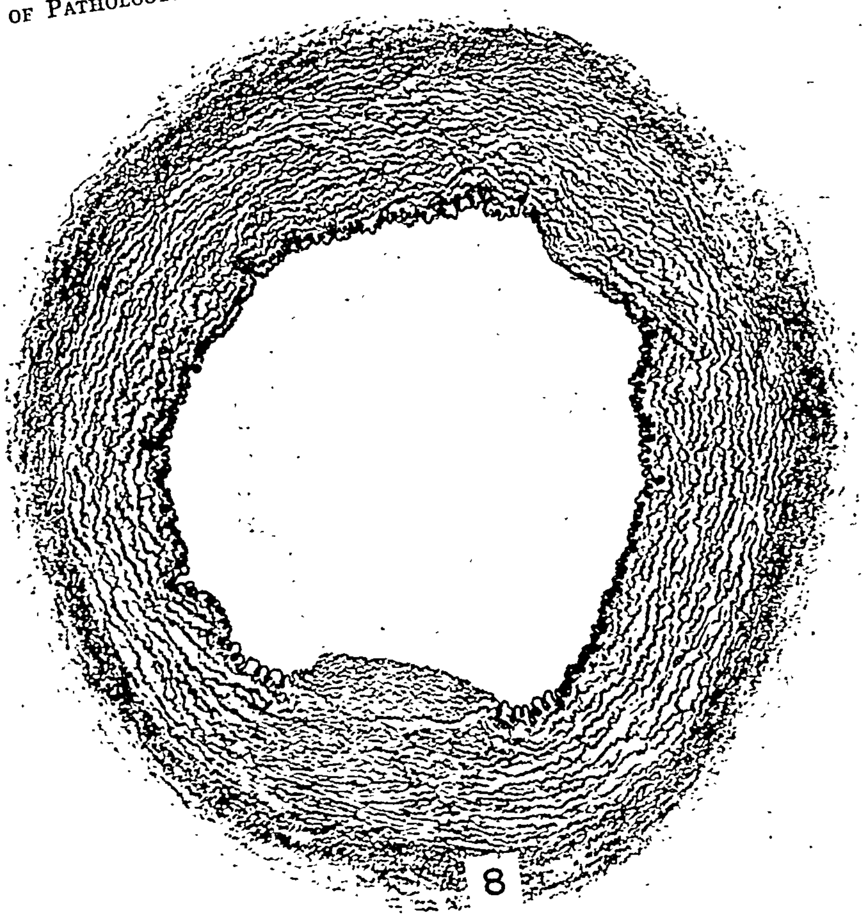

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Blood Vessels of Apparently Healthy Dogs

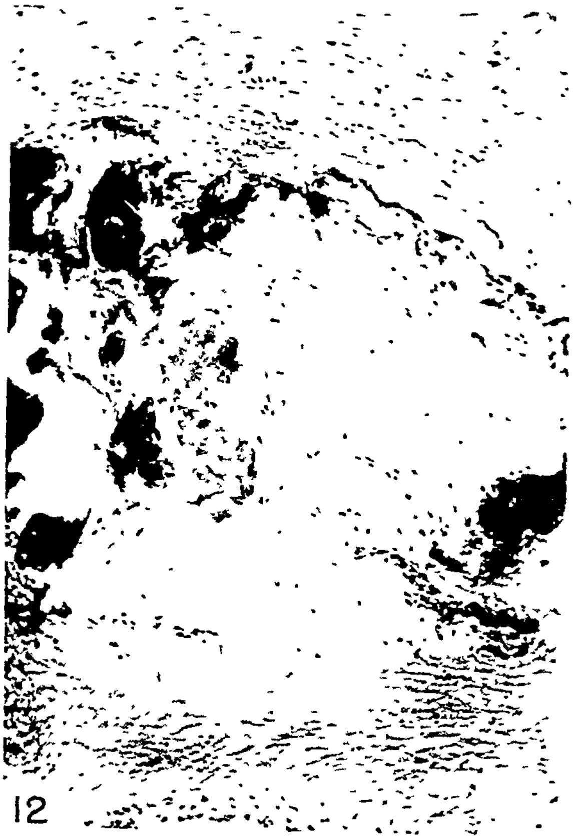
Morehead and Little

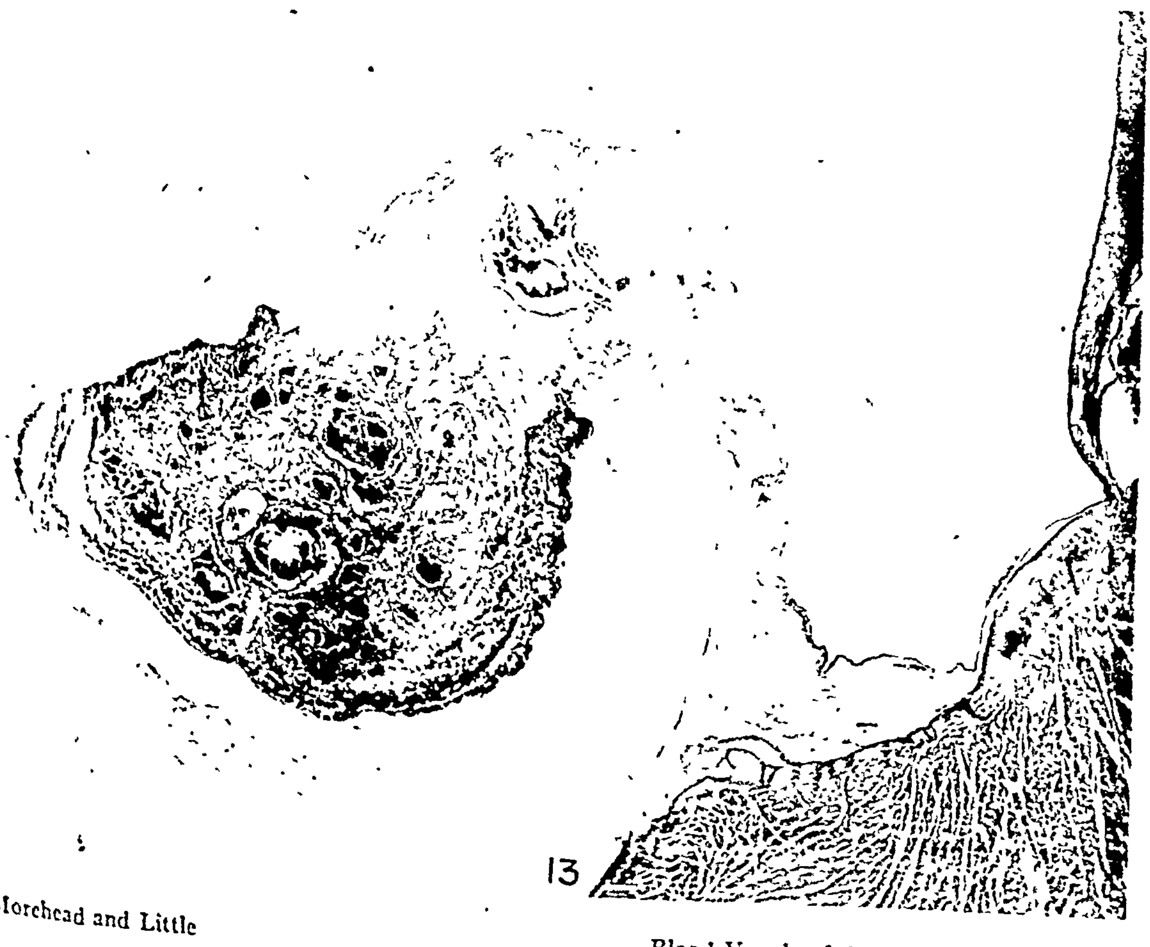
PLATE 62

FIG. 11. Calcium deposits in the intima and media of the aorta in the vicinity of the aortic valve. Masson's trichrome stain. × 60.

FIG. 12. Fibrous thickening of the intima and media of the aorta with calcification. This section was from the ascending aorta. Hematoxylin and eosin stain. × 110.

FIG. 13. Low power photomicrograph of the aortic valve showing a large vegetation. Cartilage can be seen at the base of the valve. Hematoxylin and eosin stain. × 11.





Morehead and Little

Blood Vessels of Apparently Healthy Dogs

PLATE 63

FIG. 14. Focal embolic nephritis in the dog with bacterial endocarditis. Masson's trichrome stain. × 250.

FIG. 15. Large numbers of mononuclear cells surrounding the tubules. Hematoxylin and eosin stain. × 110.

FIG. 16. Fibrous thickening of the parietal layer of Bowman's capsule. The glomerulus is surrounded by mononuclear cells. Hematoxylin and eosin stain. × 250.

FIG. 17. Destruction of renal parenchyma and replacement by fibrous tissue. There are large numbers of mononuclear cells and heavy calcium deposits. A sclerotic arteriole is seen in the lower part of the field. Hematoxylin and eosin stain. × 110.

Blood Vessels of Apparently Healthy Dogs

Morehead and Little

MYOBLASTOMA

(Granular Cell Myoblastoma or Myoblastic Myoma)*

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Tumors presumably composed of immature skeletal muscle elements and variously described as myoblastic myoma, myoblastoma, granular cell myoblastoma, rhabdomyoma, rhabdyome granulo-cellulaire, etc., are now recognized as a definite pathologic entity. Credit for the first clear description of these tumors under the name "myoblastic myoma" is given to Abrikossoff [5] who reported 5 cases in 1926. However, in 1854, Weber [1] reported a case of hypertrophy of the tongue in a male, 21 years old, who developed new growths after excision of the portion protruding beyond the teeth. These new growths presented microscopic features identical with the tumors now known as myoblastomas.

Other earlier reports of identical lesions were made by Heurtaux [2] (1881), Pendl [3] (1897) and Moschcowitz [4] (1922). The case reported by Heurtaux was that of a small nodule occurring on the dorsum of the tongue in a female, 19 years old, and was interpreted by him as granular-protein degeneration of muscle fibers. Pendl designated his neoplasm, occurring on the tongue of a male, 8 weeks of age, as a rhabdomyoma and explained it on the basis of misplaced embryonic tissue. Moschcowitz labelled his tumor, occurring on the dorsum of the tongue of a female, 30 years old, as a "xanthoma," describing it as a myogenetic form of xanthoma. Abrikossoff,[5] writing in 1926, described 5 cases (3 of the tongue and one each of the lip and gastrocnemius muscle), clearly stating their relationship to striated muscle but regarding the lesion as a degenerative process following trauma or inflammation. Later, however, in the light of cases reported by Klinge [10] and of subsequent cases of his own, he felt that some of the tumors might have developed from primitive myoblasts of the embryo.

Since Abrikossoff's report,[5] many instances of tumors of this type have been reported and we have been able to collect the details of 162 cases (Table I), including 5 of our own reported here.

Report of Cases

Case 1

The patient was a newborn white female (S. J. S37-657), delivered of a 23-year-old primipara. Physical examination was negative save for the occurrence of three distinct growths on the gingival ridge of the maxilla at about the location of the right upper lateral incisor, the largest measuring 2 by 1.5 cm. The largest

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growth was smooth, egg-shaped and pedunculated and partially covered by the smaller ones which were also pedunculated. All were covered by mucous membrane. There were no abnormal laboratory findings and roentgenograms of the bony skeleton were negative. The tumor was excised and there was no recurrence.

Grossly, the specimen consisted of four small pieces of tissue 0.7 cm. in diameter and one piece 2 by 1.5 cm. These were covered by smooth mucosa and presented firm, white to light brown cut surfaces. Microscopically (Fig. 1), the sections showed the tissue to be covered by a normal squamous epithelium and to be composed of large (30 to 70 μ), round, polyhedral, or spindle-shaped cells with acidophilic cytoplasm. The cytoplasm contained dense, coarse, acidophilic to neutrophilic granules giving it a granular appearance. The nuclei were small, round, central and vesicular, though many pyknotic forms were also present. No irregular, lobulated, or atypical tumor nuclei were noted nor were mitoses seen. No lipoidal material was identified with fat stains. The stroma was loose and edematous in some portions, but for the most part the cells were closely packed with scant supporting stroma.

Case 2

The patient was a newborn colored female (S. J. S43-643), born of a 27-year-old woman who had one previous normal pregnancy with the child living and well. The physical examination was negative save for the occurrence of a smooth red tumor covered by gingival membrane and located at the gingival margin of the mandible just to the left of the midline. The tumor was excised and the child discharged. She was returned 3 days later with a history of feeding poorly and slight bleeding from the gum. At this time the physical examination showed marked listlessness and dehydration with depression of the fontanelles. The wound in the mouth was well healed. The heart and lungs were negative. The abdomen was distended and an enlarged liver could be palpated. The extremities were negative. The child was in extremis and died 4 hours after admission.

Grossly, the tumor consisted of a nodule of tissue 1.5 by 0.8 by 0.8 cm. and covered over one surface by a smooth membrane. The cut surfaces were soft, golden-yellow and fatty in appearance. Microscopically, the tissues was composed of cords and masses of large, round, polyhedral, or spindle-shaped cells separated by a loose, edematous, fibroblastic tissue (Fig. 2). The cells had a coarsely granular, eosinophilic cytoplasm and showed small, central, round, vesicular nuclei (Fig. 3). Some of the cells were elongated, cylindrical or teardrop in shape, the longer ones showing one or two points of narrowing and containing two or three nuclei in different portions of the cell. With phosphotungstic acid and hematoxylin stain many cells showed fine, fibrillary, cross and longitudinal, purple fibrils which did not traverse the entire length of the cells (Fig. 4). The cells also showed a purple to blue, granular appearance of portions of the cell membrane.

Mallory's aniline blue stains showed similar fibrils and granular structures staining red and in a linear arrangement, and very coarse granulations staining dark purple (Fig. 3). The connective tissue extended around individual cells as fine strands of collagen but at some points the cells were in direct apposition. No mitoses were noted. Special stains for fat were negative.

The autopsy showed no evidence of tumor at the site of the original lesion. The only positive findings were in the liver and kidneys. The liver was enlarged (150 gm.) and presented a smooth yellow surface and a firm yellow cut surface. Microscopically, the liver cells were swollen and distorted by large, clear, fat droplets. The portal zones showed an apparent increase in bile ducts as seen in biliary cirrhosis. The kidneys were grossly normal but showed many fat droplets in the convoluted tubules with a special stain, but no other abnormalities. The anatomic diagnoses were: imbalance of fat metabolism with fatty metamorphosis of the liver and lipoid nephrosis, biliary cirrhosis of the liver, cerebral edema.

Case 3

The patient was a Negro, 26 years of age (S. V. 18566). A tumor the size of a walnut was removed from the anal region. Further clinical details are not available.

Grossly, the tumor measured 4 by 3 by 2.2 cm., was covered by skin and showed a red-gray, firm cut surface. Microscopically, the tumor was composed of strands and masses of cells which were polygonal to rounded and often elongated. The cells were acidophilic, finely granular, and occurred singly and in solid masses. The stroma was dense and fibrous. Special stains for fat were negative. The overlying squamous epithelium showed some downgrowth of the rete pegs and melanotic pigment had been mobilized, being present in macrophages in the papillary layer.

Case 4

A white female, 29 years old (S. V. 10324), who had injured her left knee 8 years previously, noted a mass the size of a pea on the inner side of the thigh just above the knee. This grew to the size of a small walnut. There were a few long hairs over the nodule. There was some pain in the region of the knee but the lesion proper was not tender. Physical examination was negative save for the local lesion, which was attached to the skin but not to the bone or muscle. The lesion was excised locally and there has been no recurrence.

Grossly, the tumor was 1.5 cm. in diameter, sharply circumscribed, and presented a firm, gray-white cut surface. Microscopically, the tumor was composed of groups and cords of polyhedral to spindle-shaped eosinophilic cells separated by a dense fibrous tissue. The cells

TABLE I

Cases of Myoblastoma Reported in the Literature

Year	Author	Sex	Age	Duration	Location	Size	Remarks
1854	Weber[1]	M	21	Years	Tongue	Diffuse overgrowth	
1881	Heurtaux[2]	F	19	2 years	Dorsum of tongue	Small nodule	
1897	Pendl[3]	M	8 wks.	Life	Dorsum of tongue	Pigeon's egg	
1922	Moschcowitz[4]	M	30	Months	Dorsum of tongue	1 cm.	
1926	Abrikossoff[5]	M		Months	Dorsum of tongue	0.8 x 0.5 cm.	
		F	30	1 year	Gastrocnemius	6 x 10 cm.	Local recurrence, 3 mos.
		M	25	Months	Tongue	1 cm.	
		M		Weeks	Upper lip	Cherry	Central ulceration
		M			Dorsum of tongue	0.5 cm.	
1926	Keynes[6]	M	26	9 months	Dorsum of tongue	0.5 cm.	Proliferation of epithelium
1926	Rütz[7]	M	5 mos.	Life	Dorsum of tongue	Plum	
1927	Dewey[8]	F	42	1 year	Dorsum of tongue	1.5 cm.	
1927	Diss[9]	M	59	3 months	Tongue	Pea	
1928	Klinge[10]				Tongue	Pea	
					Tongue	Pea	
					Tongue	Pea	
					Skin (2 cases)		
1928	Roffo[11]	M	16	Weeks	Tongue		
1929	Jaulin and Grandclaude[12]	F	42	Months	Dorsum of tongue	Pea	
1929	Volkmann[13]	F	Nb*	Life	Maxilla	Plum	"Congenital epulis"
1930	Diss[14]	M	38	Months	Dorsum of tongue	0.7 cm.	
1931	Abrikossoff[15]	M	37	2 months	Dorsum of tongue	0.5 cm.	
		M	21	1 year	Skin	1.5 cm.	
		M	24	Years	Vocal cord	Pea	
		F	55	2 months	Breast	2 x 1.5 cm.	
		F	29		Esophagus	Pea	
		M	36	1 year	Mandible	Fist	Giant myoblasts
		M	55	10 years	Mandible	Hen's egg	Giant myoblasts
1931	Bolanos and Despaigne[16]	F	12	Months	Tongue	Pigeon's egg	Hyperkeratosis of epithelium
1931	Ceelen[17]	M	50	3–4 years	Tongue	0.75 cm.	Proliferation of epithelium
		F	8 da.	Life	Maxilla	Pea	"Congenital epulis"; mitoses of tumor cells
		F		1½ years	Skin, pelvis	Pigeon's egg	
			Nb*	Life	Maxilla	Bean	"Congenital epulis"
1931	Dawydow[18]	M	30	1 year	Vocal cord	Pea	Proliferation of epithelium, resembling early carcinoma

1931	*Derman and Golbert*[19]	M	40	1 year	Tongue	0.8 x 1.3 cm.	Mitoses
		F	32		Tongue	Small nodule	Mitoses
		M	30	1 year	Vocal cord	Lentil	Proliferation of epithelium
1932	*Meyer*[20]		Nb*	Life	Maxilla	4.0 x 6.0 x 9.0 cm.	No recurrence, 2 years
		F	23		Breast	Walnut	No recurrence, 6 months
		M	69	3 weeks	Tongue	0.4 x 0.8 cm.	No recurrence, 3 months
1932	Schirmer[21]	M	36	7 weeks	Tongue	Hazelnut	Overlying squamous cell carcinoma
1933	Glasunow[22]	M	32	4 months	Dorsum of tongue	1.0 cm.	
1933	Roffo[23]	F	31	3 years	Dorsum of tongue	Small nodule	
1934	Geschelin[24]	F	40	Months	Vocal cord	Pea	
1934	Geschickter[25]				5 of muscles of extremities		Details not given
					3 of breast		
					3 of subcutis in sacral region		
					Ear		
					Lip		
					Tongue		
1934	Kleinfeld[26]	M	50	2 years	Vocal cord	1 cm.	Epithelial proliferation
1934	Klemperer[27]	M	58	20 years	Skin, groin	Pecan	Well, 8 months
		F	23	1 year	Skin, pubis		Well, 2 years
		M	50	2 years	Vocal cord	1 cm.	Same case reported by Kleinfeld
		M	40	6 months	Tongue	Pea	Epithelial proliferation
		M	41	Years	Skin, thigh	Cherry stone	Epithelial proliferation
		F	42	12 years	Calf	7.5 cm.	Well, 1 year
1935	Gander[28]	F	52	6 months	Dorsum of tongue	Pea	
1935	Kernan and Cracovaner[29]	F	21	3 months	Vocal cord	Pea	No recurrence, 10 months
1935	Martinez[30]	F	50	18 years	Dorsum of tongue	1.5 cm.	
1935	Morpurgo[31]	F	65	Months	Tongue	Small nodule	Mitoses: carcinoma of overlying epithelium
					Tongue	Small nodule	
					Muscle, extremity		
1935	Seiffert[32]	M	47	Months	Dorsum of tongue	Pea	
1936	Bobbio[33]				Tongue		
1936	Lattes[34]				Tongue		
1936	Parreira and Nunes de Almeida[35]	F	16		Tongue	Small nodule	
		F	51		Tongue	Small nodule	

Table continued on following pages.

* Nb indicates newborn infant, age not specified.

TABLE I—*continued*

Year	Author	Sex	Age	Duration	Location	Size	Remarks
1937	Bang[36]	M	63	2 years	Dorsum of tongue	Small nodule	Epithelial proliferation; well, 5 years
1937	Cappell and Montgomery[37]	M	61	9 days	Bladder	2.5 cm.	
		F	27	Months	Urethral meatus	Nutmeg	No recurrence, 6 years
		M	52		Spermatic cord	6.0 x 4.5 cm.	
1937	Gray and Gruenfeld[38]	F	30	5 months	Tongue	Hazelnut	No recurrence, 9 years
		M	35	6 months	Tongue	0.6 cm.	No recurrence, 6 years
		F	55	Weeks	Skin, breast	1.5 cm.	
		F	30	1½ years	Tongue	Pea	No recurrence, 3 years
		F			Breast	10.0 cm.	
1937	Szathmary[39]	F	32	Months	Skin, perineum		
1938	Ducuing, Ducuing and Bassal[40]	M	34	20 months	Dorsum of tongue	Pea	Epithelial proliferation; well, 6 months
		M	52	2 months	Dorsum of tongue	Pea	Epithelial proliferation; well, 1 year
		M	22	2 months	Dorsum of tongue	1.5 cm.	Epithelial proliferation; well, 1 year
		M	44	6 months	Dorsum of tongue		Well, 6 months
1938	Frenckner[41]	M	25	1 week	Arytenoid	3.5 x 0.75 cm.	Cross and longitudinal striations; no recurrence in 2½ years
		F	28	1 year	Trachea	Hazelnut	No recurrence, 3 years; also reported by Bergstand
		M	41	3 years	Lower lip	1.0 x 1.0 x 1.3 cm.	
		F	22	3 years	Tongue	Small nodule	
		M	48	1 month	Tongue	Pea	
1938	von Bahr[42]	F	49	Years	Lacrimal sac	0.4 cm.	
1939	Civatte and Ducourtioux[43]	F	36		Tongue	Small nodule	
1939	Dustin[44]				Tongue		
1939	Eickhoff[45]	M	25	6 months	Tongue		Overlying squamous cell carcinoma
1939	Fräsdorf[46]	M	68		Pharynx	2 small nodules	
1939	Geschickter and Maseritz[47]	F	62		Muscle, arm		Well, 14 months
		F	45	1 year	Muscle, pectoral		Well, 14 months
		M			Muscle, arm		Well, 4 years
		M			Muscle, thigh		No recurrence
					Subcutis, sacrum		No details given
					Subcutis, sacrum		No details given
1939	Kramer[48]	F	15	14 weeks	Bronchus		
1939	Leroux and Delarue[49]	M	37	3 months	Tongue	1.5 cm.	Epithelial proliferation
		M	45	3 months	Tongue	Pea	
1941	Grayzel and Friedman[50]	M	Nb*	Life	Maxilla	1.0 x 0.5 cm.	
1941	Kratochvil[51]	F	45	Months	Axilla	Walnut	
1941	Lascano-Gonzalez[52]				Maxilla		
		M	26	15 days	Tongue	1.2 x 0.8 x 0.7 cm.	
			Nb*	Life	Maxilla	1.2 x 0.9 x 0.8 cm.	

1941	Tamis and Kowles[53]	F	39	3 years	Labia	Small lump	
1941	*Thoma*[54]	F	30	1½ years	Tongue	1.5 cm.	
1942	*Iglauer*[55]	F	41	1½ years	Arytenoid	Hazelnut	
1942	*Monteiro*[56]				Tongue		
1942	Tuta and Schmidt[57]	F	40		Breast	1.0 x 0.7 x 0.5 cm.	
		F	69	3 years	Skin, iliac	2.5 x 2.0 x 1.0 cm.	
		F	7		Skin, sternum	0.8 cm.	
		M	47	3 years	Skin, shoulder	3.0 x 2.5 x 2.0 cm.	
1943	Altmann[58]	M	60	20 years	External auditory canal		No recurrence, 18 months
		F	77	5 years	External auditory canal		
		F	27	5 years	External auditory canal		
1943	Horn and Stout[59]	F	60	20 years	Ext. auditory canal		Also reported by Altmann
		F	77	5 years	External auditory canal		Also reported by Altmann
					5 of tongue		
					1 of lip		
					2 of maxilla or mandible		
					1 of floor of mouth		
					1 of trachea		
					3 of skin		
					10 of subcutis		
					1 of muscle, extremity		
					2 of breast		
					1 of vocal cord		Also reported by Kernan and Cracovaner
1894	Massin[60]	F	Nb*	Life	Maxilla†		2 nodules; large granular cells
1929	Kleine[61]	F	Nb*	Life	Maxilla†		
		F	Nb*	Life	Maxilla†		No recurrence, 1½ years
1932	Jorge, Nudelman and Gringauz[62]	F	Nb*	Life	Mandible†		Large granular cells; well, 3 years
		F	Nb*	Life	Mandible†		Large granular cells
		F	Nb*	Life	Mandible†		Large granular cells

* Nb indicates newborn infant, age not specified.

† Recorded as congenital epulis of newborn.

We believe that the case reported by Imperatori[63] as a rhabdomyoma belongs in that group, although others have included it with the myoblastomas. Cases reported by Lino,[64] v. Meyenburg,[65] Giardino,[66] Cooper[67] and Bailey[68] were examined and discarded. Cases reported by Kraneis,[69] Lepage,[70] Jorge and Brachetto Brian[71] and La Manna[52] were not available to us.

were finely granular and had small vesicular nuclei. Mitoses were not noted. A special stain for fat was negative.

Case 5

The patient was a white male, 47 years old (S. V. 13400), who complained of a hard spot on his tongue of 2 months' duration. There was no history of injury. The physical examination was negative save for the occurrence of a firm, bean-like nodule at the lower right margin of the tongue. It was not ulcerated. The lesion was excised locally without recurrence.

The gross findings are not known. Microscopically, the lesion was composed of masses of large, granular, polyhedral to ribbon-like cells intermingled with skeletal muscle. The nuclei were small and vesicular. No mitoses were seen. At some points adult skeletal muscle cells ended in bulbous granular swellings similar in appearance to the cells composing the major portion of the lesion.

Morphologic Features

Gross Characteristics. The tumors reported as myoblastomas average 1 to 2 cm. in diameter, the largest one being 10 cm. in diameter, and the majority are sharply circumscribed or truly encapsulated. They may, however, be poorly demarcated and irregularly penetrate the underlying tissues. Those located under the mucous membrane are usually covered by a smooth, intact epithelium, although some reported cases have shown ulceration of the epithelium and others hyperkeratotic, or even malignant proliferation of the epithelium. They may be nodular or lobulated but usually present a solitary, distinct, round mass. Frequently they are pedunculated, polypoid, or sessile. On section they have firm, gray to yellowish-gray or tan surfaces.

Microscopic Characteristics. The tumors are composed of large polyhedral cells, the majority being irregularly rounded, but transitional forms having a tear-drop, spindle, or cylindrical shape may also be present. The rounded forms vary from 20 to 60 μ in diameter, while the spindle or cylindrical forms are much longer, measuring up to 300 μ in our own cases. Under low magnification the cells appear to occur singly or in groups, but with higher magnifications and special stains for connective tissue, such as Mallory's aniline blue, Masson's trichrome or van Gieson's, delicate strands of connective tissue are found to penetrate among and surround the majority of the cells, but small clusters of cells do occur in direct apposition to one another. In some portions the collagen framework is abundant and edematous in polypoid lesions.

The most characteristic feature is the cytoplasm of the cells which is abundant, acidophilic and granular. The granules vary from the fine acidophilic to large, coarse forms that are neutrophilic. It must be em-

phasized that the cells are granular and *not* vacuolated and that special stains for lipoid are negative. Some authors [72,73] have suggested that the cells may contain glycogen and mucin-like substances but adequate studies with proper fixation have not been done to warrant any statement in regard to these substances. Longitudinal and cross striations have been demonstrated but not constantly, and Klemperer[27] has described an arrangement of the granules in rows resembling fibrils. With immediate proper fixation, and staining (Zenker's and Mallory's phosphotungstic-acid-hematoxylin and aniline blue stains) our own cases show clear-cut, fine, longitudinal and cross fibrils.

The nuclei are small (7 to 10 μ) and vesicular, but pyknotic forms may be numerous. They are round to oval and centrally located. In our own cases some of the longer, cylindrical forms contain 2 or 3 nuclei. Mitoses are usually absent although Ceelen,[17] Derman and Golbert [19] and Morpurgo [31] reported their occurrence.

An additional associated finding is the proliferation of the overlying epithelium first mentioned by Keynes,[6] noted by many others and emphasized by Klemperer.[27] Some cases of hyperkeratosis of the epithelium have been reported and cases reported by Schirmer,[21] Morpurgo [31] and by Eickhoff [45] showed squamous cell carcinomas of the overlying epithelium, while Leroux and Delarue [49] reported a case associated with a basal cell carcinoma.

Differential Diagnosis. These tumors are most likely to be interpreted as xanthomas, the first two cases reported here having been so diagnosed originally and case 1 so reported in the literature.[74] The total absence of fat differentiates them from the true xanthomas. Geschickter [25] reported 2 cases that were misinterpreted as chordomas. So-called congenital epulis of the newborn, as described by Massin,[60] Kleine [61] and Jorge, Nudelman and Gringauz,[62] presents features identical with the myoblastomas and we believe that they should be so regarded. The tumors are also to be differentiated from rhabdomyomas and rhabdomyosarcomas which are composed of adult skeletal muscle elements.

Clinical Features

Incidence as to Age and Sex. These tumors occur at all ages and with equal frequency among males and females, as shown in Table II; they are not more common in males as previously supposed. Forty-seven per cent of the cases have occurred in persons under the age of 50 years, with the greatest incidence occurring in the third, fourth and fifth decades. Lesions of the alveolar processes occur chiefly in children. All of the maxilla were in newborn infants, but two of three tumors of the mandible occurred in the third and fifth decades.

Distribution. The location of the tumors in various portions of the

body is indicated also in Table II. The tongue is by far the most common site, 38 per cent of reported cases occurring in this location and the vast majority being located on the dorsal surface. Skin, subcutaneous tissue, muscle, alveolar processes, respiratory tract, breast, ear, and lip, in decreasing order of frequency, account for the majority of the other cases. The skin lesions are reported from the pelvic region, extremities and breast. Isolated instances have also occurred in the anus, bladder,

Table II

Location, Age and Sex Distribution of 162 Cases of Myoblastoma

Age in decades	1–9		10–19		20–29		30–39		40–49		50–59		60–69		70–79		Totals			
Sex	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	Age and sex not given	Final
Alveolar process																			2	2
Mandible		4					1				1						2	4		6
Maxilla	1	6															1	6	4	11
Breast						1						1						2	6	8
Ear						1								1		1		3		3
Larynx and vocal cord					2	1	2			2	1						5	3		8
Lip									1								1		3	4
Muscle								1		2				1				4	9	13
Skin		1			1	1		1	3		1	1		1			5	5	7	17
Subcutis						1				1								2	15	17
Tongue	2		1	3	6	1	8	6	7	2	3	3	2	1			29	16	16	61
Trachea and bronchi				1		1												2	1	3
Other*					1 A	2 EU		1 V		1 L	1 S		2 BP				4	4	1 M	9
Totals	3	11	1	4	10	9	11	9	11	8	7	5	4	4	0	1	47	51	64	162

* A = anus, B = bladder, E = upper esophagus, L = lacrimal sac, M = floor of mouth, P = pharynx, S = spermatic cord, U = urethra, V = vulva.

This table includes our own cases and those collected from the literature, as presented in Table I.

esophagus, lacrimal gland, floor of the mouth, pharynx, spermatic cord, urethra and vulva. Stout [75] has recently encountered 2 examples in the uterus. While more than one nodule may occur at one site, there are no instances of tumors occurring at more than one place in one patient.

Lesions. The tumors occur as firm, small (1 to 2 cm.) nodules that may be polypoid or sessile. They may increase slowly in size. The overlying epithelium is usually intact but may be ulcerated. They are nontender.

Course. The lesion is a benign one and local excision will effect a complete cure. However, if incompletely removed they may recur locally, as reported by Horn and Stout; [59] and Geschickter [25] reported a tumor starting in the sacral region which showed metastases to the groin. The metastases were treated with radium and the patient was

well 20 months later. Frankly malignant tumors, as in the cases of v. Meyenburg,[65] should be more properly classified with the rhabdomyosarcomas. The longest reported duration prior to medical attention was 20 years.

Discussion

The new cases recorded here are identical with the previously recorded cases of myoblastoma, being composed of large cells with a coarsely granular cytoplasm and small vesicular nuclei. Of particular significance are the various cellular forms as noted in case 2 in which cylindrical cells were found, and the demonstration of definite longitudinal and cross fibrils. Figure 4 is a composite camera lucida drawing showing the various types of elongated cells and the fibrils.

There has been much contention as to the nature of these tumors. One group believes them to be true neoplasms composed of immature, proliferating skeletal muscle cells (myoblasts), while others regard the lesions as degenerative, interpreting the cells as muscle fibers undergoing necrobiotic changes. Abrikossoff,[5] in his first paper, regarded the tumors as resulting from a degenerative process following trauma or inflammation in striated muscle, but in reporting his second series of cases,[15] in 1931, he stated that some of the tumors might originate from primitive myoblasts representing embryologic rests (Cohnheim), as suggested by Klinge [10] and even earlier by Pendl [3] and Rütz.[7]

The concept that these lesions are composed of proliferating myoblasts has been based upon the morphologic resemblance of the cells to immature skeletal muscle fibers as described by Godlewski.[76] He described myoblasts as showing granules occurring in rows and later the formation of homogeneous fibrils. Wolbach,[77] in 1928, in studies of rhabdomyomas of the heart and breast, again noted the linear arrangement of the cytoplasmic granules and believed that the myofibrils were formed by the connection of these granules which, he stated, develop from the multiplication and dispersion of centrioles and centriole clusters. Klemperer [27] described a similar arrangement of granules in one of his cases, and this is also evident in our case 2. The occurrence of the cross striations has also supported the myogenous nature of these tumors, and Geschickter [25,47] as well as others has placed much emphasis on the occurrence of longitudinal fibrils. The development of the tumors in sites apart from muscular tissue is presumably due to the close embryologic association of the skin and muscular anlage. According to Maximow and Bloom,[78] the lower part of the medial layer of each primitive segment of the embryo forms the sclerotome while the lateral layer of the segment produces the skin plate. The remaining portion of the medial layer, the myotome, represents the primordium

of the dorsal and ventral musculature of the body. The occurrence of various skin tumors has been explained on the basis of imperfect separation, muscular elements being carried in the skin plate. Pendl [3] was the first to suggest the development of these myomas from misplaced embryonal tissues and subsequently the hypothesis of their development from embryologic rests has been embraced by many authors.

The original concept of Abrikossoff [5] that these lesions are a degenerative process of skeletal muscle has been championed particularly by Gray and Gruenfeld,[38] who demonstrated granular cells in degenerating skeletal muscle cells in a nonspecific ulceration of the tongue. They object to the designation of these tumors as myoblastomas on their presumed resemblance to the myoblasts described by Godlewski [76] and cite the works of Zechel,[79] Franz [80] and Marcus [81] on myogenesis, which make no reference to granular forms. Furthermore they were unable to demonstrate granular cells in embryos of man and laboratory animals. The transitional forms so frequently seen in the tongue they regard as skeletal muscle cells undergoing necrobiotic changes. Heurtaux,[2] Abrikossoff,[5] Roffo,[11] Gander,[28] Martinez [30] and Civatte and Ducourtioux [43] were all impressed by the probable degenerative nature of these lesions. Gray and Gruenfeld, and Gander suggest that all of the tumors described as myoblastomas are not histogenetically identical, regarding the lingual lesions as due to necrobiosis, while leaving the exact nature of the various other tumors open to question. This applies to our own cases, as the lesion of the tongue shows intermediate forms suggestive of muscle degeneration while this was not evident in any of the other lesions. The fact that the myoblastomas are stationary or slow-growing while striated muscle tumors are usually highly malignant is also used as an argument against the neoplastic nature of the myoblastomas.

At the present time conclusions as to the histogenesis of these lesions are not warranted. It may well be that some represent true neoplasms while others, as those of the tongue, are the result of a local degeneration of skeletal muscle.

Summary

From the literature 157 cases of myoblastoma have been collected, to which are added 5 of our own, occurring in the maxilla, mandible, tongue, subcutis and anus.

The tumors occur with equal frequency in both sexes, in all age groups, and are widely distributed over the body.

Histologically identical tumors occur in sites containing skeletal muscle and also at points normally showing no skeletal muscle elements. The most common site is the tongue and mouth, but lesions occur in other portions of the body as well.

Myoblastomas are benign tumors and complete local excision will effect a complete cure.

Definite conclusions as to the histogenesis of the myoblastomas are not warranted at the present time, the evidence being equally well founded for the myoblastic nature of some tumors and the degenerative nature of others.

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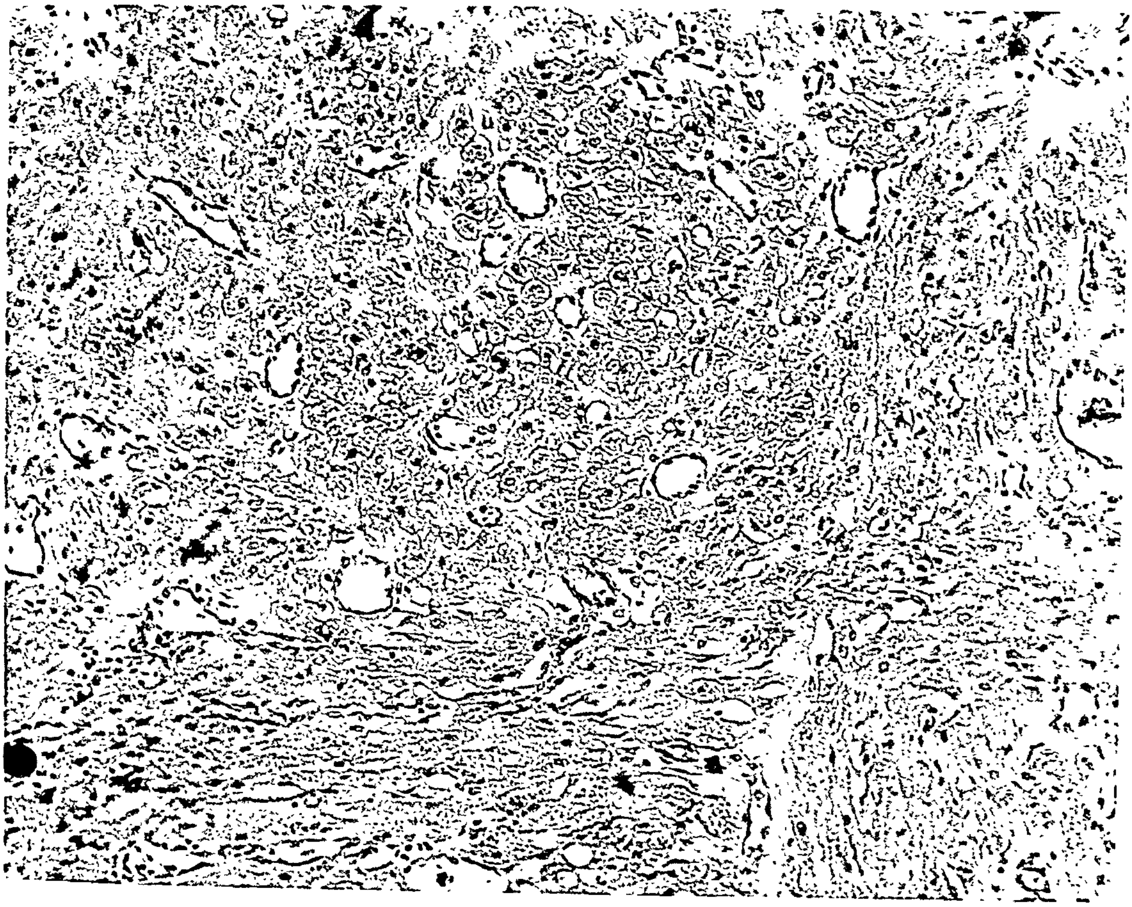
DESCRIPTION OF PLATES

Plate 64

Fig. 1. Case 1. Tumor of the maxilla showing the large polyhedral to cylindrical, granular cells. Hematoxylin and eosin stain. × 150.

Fig. 2. Case 2. Tumor of the mandible showing polyhedral to cylindrical, granular cells identical with those shown in Figure 1. Some forms show more than one nucleus. Mallory's aniline blue stain. × 150.

1



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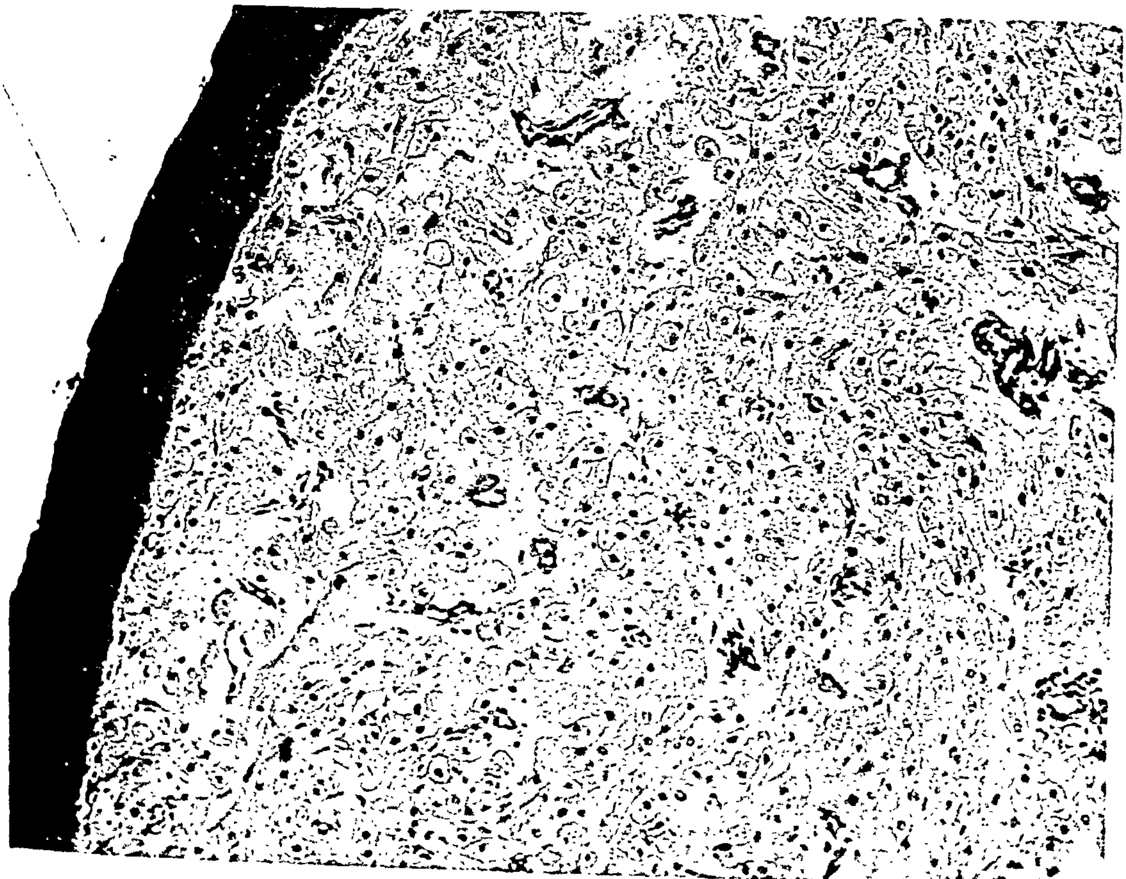


Crane and Tremblay

Myoblastoma

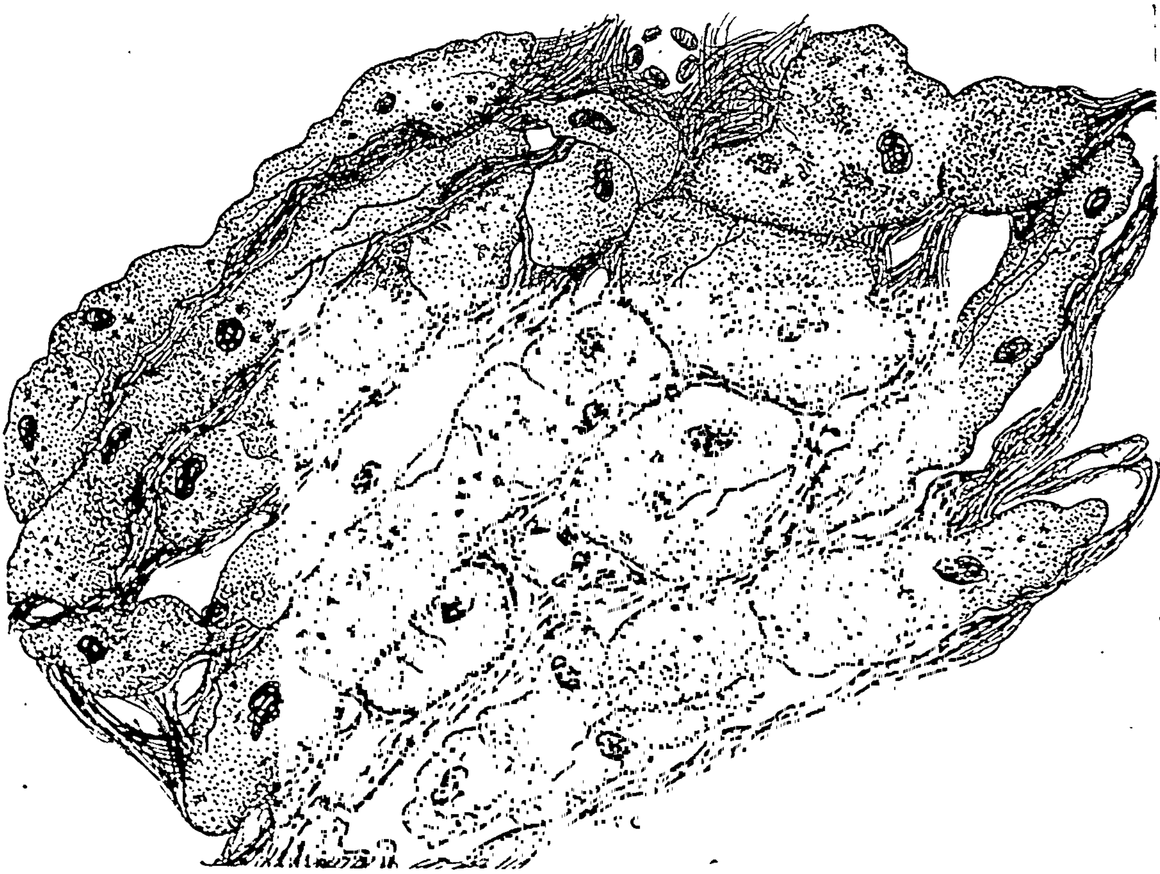
PLATE 65

FIG. 3. Case 2. Same field as that shown in Figure 2 but at a higher magnification, showing the coarse granularity of the cells and the irregular fibrils traversing the cells. Mallory's aniline blue stain. × 675.

FIG. 4. A composite camera lucida drawing of case 2 showing the detail of the longer cylindrical forms with multiple nuclei and fibrils. × 700.

3

4



VACCINAL INFECTION OF THE CHORIOALLANTOIC MEMBRANE OF THE TURTLE EMBRYO *

Paul N. Harris, M.D.
(*From the Lilly Research Laboratories, Eli Lilly and Co., Indianapolis, Ind.*)

The description in 1931 by Woodruff and Goodpasture [1] of infection of the chorioallantoic membrane of the chick embryo by fowl-pox virus introduced a new method of investigation of viral infections. Their technic has been applied so fruitfully to the study of other viruses that a vast literature has accrued during the past 13 years.

The experiments hitherto reported have been performed solely upon avian embryos. It seemed not unreasonable to suppose that attempts to induce viral infection of membranes of reptilian embryos might yield additional information.

Method

Eggs of two species of turtles, *Pseudemys troostii* and *Chrysemys picta dorsalis* were candled, and only those were used in which the embryos appeared to be in an intermediate stage of development. The shell over the back of the embryo was painted with an antiseptic solution, and after several minutes the shell was scraped away with a scalpel in a region about 2 mm. in diameter. More antiseptic solution was then applied to the exposed shell membrane, and after another pause to permit germicidal action, the membrane was punctured by a 24 gauge hypodermic needle and the suspension of virus was injected. After inoculation, the defect in the shell was sealed by collodion, the eggs were embedded in moist sand and covered with damp absorbent cotton. Eggs which were inoculated with vaccine virus were kept at 37° C., and those which were inoculated with fowl-pox virus were kept at room temperature (approximately 28° to 35° C.).

Because of turgescence of the eggs and absence of an air sac, the technical difficulties encountered were great, and it must be admitted that turtle eggs are not ideal for this type of experiment. Approximately 1500 eggs were obtained for this investigation, but only 46 were actually inoculated. Many eggs were infertile, others contained dead embryos, and many had reached such a late stage of development as to be no longer usable. Frequently, upon puncture of the shell membrane, the shell split almost explosively over half or more of the length of the egg, and even if the shell failed to split, albumin invariably exuded through the perforation in the shell membrane, as much as 0.5 to 1 cc. being lost. Aspiration of an additional 0.2 to 0.5 cc. of albumin was performed on some eggs before the virus was injected. An

* Received for publication, April 20, 1944.

attempt to obviate splitting of the shell by placing the eggs in hypertonic (10 per cent) NaCl solution for 10 to 15 minutes before treatment met with some success, but this did not prevent leakage of albumin after puncture of the shell membrane. It seems probable that this maneuver succeeded largely because it wet the shell thoroughly. Furthermore, if the eggs were allowed to become dry the shells split spontaneously.

Vaccine Virus

Twenty-one eggs were inoculated with commercial vaccine virus. Undiluted virus was used for 2 eggs, and for all others the virus was diluted with from 1 to 5 volumes of normal NaCl solution. The volume of the inocula ranged from 0.01 to 0.6 cc. Sixteen eggs were opened and examined 2 days after inoculation, 3 were opened 3 days after inoculation, and 2 were examined 5 days after inoculation. Membranes were fixed in Zenker's fluid and stained by Perdrau's [2] modification of Mann's stain.

No lesions were found in the membranes of 8 eggs. In 6 eggs there was a small hole in the chorioallantoic membrane surrounded by slight thickening, and in the remaining 7, single or multiple opaque white spots were found in the chorioallantoic membrane. Microscopic examination revealed, in the membranes from 10 eggs, proliferation and hypertrophy of ectodermal cells alone, or of both ectodermal and entodermal cells, sometimes with leukocytic infiltration of mesoderm and ectoderm. This reaction could be attributed to the presence of the virus only in eggs nos. 3 and 8, which were incubated for 5 days after inoculation, since Guarnieri bodies were not seen in membranes from any of the other eggs.

The membrane from egg no. 8 contained a lesion 2 by 4 mm. in diameter. Sections revealed thickening of both ectoderm and entoderm by cell proliferation and hypertrophy, and thickening of the mesoderm with but little cell proliferation. There was moderate leukocytic infiltration of ectoderm and mesoderm. A region of slight ectodermal ulceration was seen. Large, finely granular Guarnieri bodies were present in the cytoplasm of many ectodermal cells, and some cells contained two inclusions. Mitoses were easily found in both ectoderm and entoderm. The membrane from egg no. 3 contained multiple small lesions. Some sections were, in general, similar to those of egg no. 8, although Guarnieri bodies were less abundant. Other sections showed ectodermal ulceration and much leukocytic infiltration and fibroblastic proliferation in the mesoderm.

Six alligator eggs were inoculated with 0.05 cc. each of undiluted commercial vaccine virus after windows had been made in the shell and shell membrane. Two days later 3 embryos were dead, and the membranes of the other 3 had become infected as a result of bacterial contamination. Sections of the membranes showed

heavy leukocytic infiltration, and no Guarnieri bodies. Because of the opacity of the shell which makes candling very difficult, the absence of an air sac, and the tendency of the shells to crack on drying, these eggs are ill suited to this type of experiment.

Fowl-pox

Twenty-five turtle eggs were inoculated with from 0.05 to 0.4 cc. of fowl-pox virus suspension. This was prepared by grinding the membrane from an infected chick embryo in 1.5 cc. of 50 per cent glycerine and diluting with 3 cc. of normal NaCl solution. The eggs were examined 4 to 6 days later. At this time 7 embryos were dead and 5 were hatching. The membranes of 4 showed no gross lesions, and the membranes of 9 showed slight thickening. Sections contained no inclusions, although slight ectodermal and entodermal proliferation and leukocytic infiltration of the mesoderm were seen.

Discussion

It was our original design to observe the effects of several viruses upon the embryos of turtles, alligators, and snakes, but the experiments which had been planned could not be carried out on the intended scale because of remoteness from the source of supply, and because of the short seasonal limitation on the availability of eggs. Although several dozen eggs of different species of snakes were obtained, none could be inoculated because of infertility or death of the embryos before arrival at the laboratory. The results are presented now since work cannot be resumed here, and with the hope that others who have at hand a source of supply of reptilian, amphibian and piscine eggs will be thereby encouraged to take up the investigation and will find herein helpful ideas as to procedures.

The low incidence of vaccinal infection observed in this experiment was doubtless due to the difficulty of inoculation. The procedure was a blind one, and it was impossible to be sure that the inoculum was placed upon the chorioallantoic membrane. In fact, it is highly probable that most inoculations were made into the allantoic cavity or into the albumin sac. Creation of an artificial air sac over the chorioallantoic membrane would obviate this difficulty. However, demonstration of Guarnieri bodies in the chorioallantoic ectodermal cells of 2 eggs indicates the susceptibility of membranes of turtle embryos to vaccinal infection. In view of the susceptibility of membranes of chick embryos to infection by a wide variety of viruses, and to the susceptibility of the cornea and skin of young turtles to vaccinal infections as shown by Andervont,[3] it is to be expected that the membranes of turtle embryos would be susceptible to vaccinia.

The inclusions observed in the membranes of turtle embryos are quite similar to those which develop in the membranes of chick embryos. Differentiation of leukocytes from Guarnieri bodies is easy since

the latter are finely granular, lie within the cytoplasm of the ectodermal cells and are surrounded by a clear zone which in some cells is quite broad.

Failure of the eggs inoculated with fowl-pox virus to develop inclusions is not interpreted as indicating lack of susceptibility. However, it is stated by Findlay [4] that the tortoise and frog are resistant to fowl-pox, and it may be that membranes of turtle embryos are also resistant.

Summary

1. The chorioallantoic membrane of the turtle embryo reacts to the presence of vaccine virus by the formation of finely granular Guarnieri bodies.

2. The technic of inoculating turtle eggs and the difficulties encountered are described.

I am indebted to Dr. Clinton L. Baker, Director of the Reelfoot Lake Biological Station of the Tennessee Academy of Science, for obtaining the turtle eggs, and to Dr. E. W. Goodpasture of Vanderbilt University for an expression of opinion as to the nature of the inclusions found in the membranes.

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DESCRIPTION OF PLATE

Plate 66

In order to facilitate identification of Guarnieri bodies in these illustrations, the distance of the center of the inclusion from the right margin and upper margin of the prints respectively will be given in millimeters.

Fig. 1. Egg. no. 8. Six Guarnieri bodies are seen at 116 and 26, 109 and 11, 72 and 7, 54 and 7, 15 and 43, and 8 and 38. The third and fourth lie at opposite poles of the nucleus of a single ectodermal cell. Granularity of the inclusions is obvious. × 620.

Fig. 2. Egg no. 8. Twelve Guarnieri bodies are seen at 93 and 7, 93 and 17, 61 and 12, 61 and 26, 53 and 15, 49 and 9, 45 and 13, 43 and 17, 37 and 9, 16 and 10, 26 and 20, and 14 and 20. The last two lie at opposite poles of the nucleus of a single ectodermal cell. Several leukocytes with large black cytoplasmic granules are seen in the thickened ectoderm. × 620.

Fig. 3. Egg no. 8. Guarnieri bodies are seen at 19 and 27, and 17 and 58. × 1200.

Fig. 4. Egg no. 8. Guarnieri bodies are seen at 22 and 9, and 22 and 65. A leukocyte is seen at the right center. × 1200.

Fig. 5. Egg no. 3. Guarnieri bodies are seen in adjacent cells at 23 and 32, and 23 and 42. × 1200.

1

2

3 4 5

Harris

Vaccinal Infection of Chorioallantois of the Turtle

MULTICENTRIC BILATERAL CARCINOMA OF THE KIDNEYS *

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The case described herein is reported because it presents an unusual if not a unique variety of primary carcinoma of the kidney. Both kidneys were the site of multiple small tumors arising from the tubules, and giving rise to extensive metastases. As far as could be determined, a similar case has not been previously recorded.

The patient was a white woman, 65 years of age, with mild diabetes mellitus whose history and course suggested a malignant growth, the origin of which could not be determined clinically. Pyelonephritis and hematuria were discovered during cystoscopic examination.

Necropsy was performed 3 hours after death. The most conspicuous finding was marked lymphadenopathy of the abdominal and thoracic nodes and small nodules in the pleura, lungs, adrenals, ileum, stomach and kidneys. The nodes were enlarged, soft, gray-white and showed a few small hemorrhages. The node at the hilum of the liver was firmly adherent and locally invasive. The kidneys were enlarged: the right weighed 250 gm.; the left, 240 gm. The capsules stripped easily and the cortical surfaces were smooth. The organs were studded with small gray-white masses measuring 1 to 2 cm. in diameter. The masses in the stomach and ileum were covered with normal mucosa and in the latter organ were located in the tips of the valvulae. The anatomic diagnosis was lymphosarcoma with metastases.

The histologic study of the renal masses showed tumors in which the normal pattern was well preserved. They consisted of tubules separated by capillaries and lined by a single layer of large opaque cells which varied moderately in size. The nuclei were large and hyperchromatic, and mitoses were frequent. An occasional glomerulus was enmeshed at the periphery. In one section, a glomerulus was found which showed a layer of tumor cells lining the capsular basement membrane and a solitary tumor cell lying on the glomerular basement membrane. There was slight evidence of compression of the adjacent renal parenchyma but occasionally a tubule extended like a finger into the normal tissue. The tumors of the other organs were identical carcinomas. The final diagnosis was multiple primary carcinoma of the kidneys with metastases to the abdominal and thoracic lymphatics, pleura, lungs, adrenals, ileum and stomach.

* Received for publication, April 17, 1944.

DESCRIPTION OF PLATE

Plate 67

Fig. 1. Photomicrograph of a renal tumor showing the tubular structure separated by a capillary network. $\times$ 90.

Fig. 2. Photomicrograph showing the type of tumor cell lining the tubules. $\times$ 400.

Fig. 3. Photomicrograph showing a glomerulus with malignant capsular epithelium. $\times$ 400.

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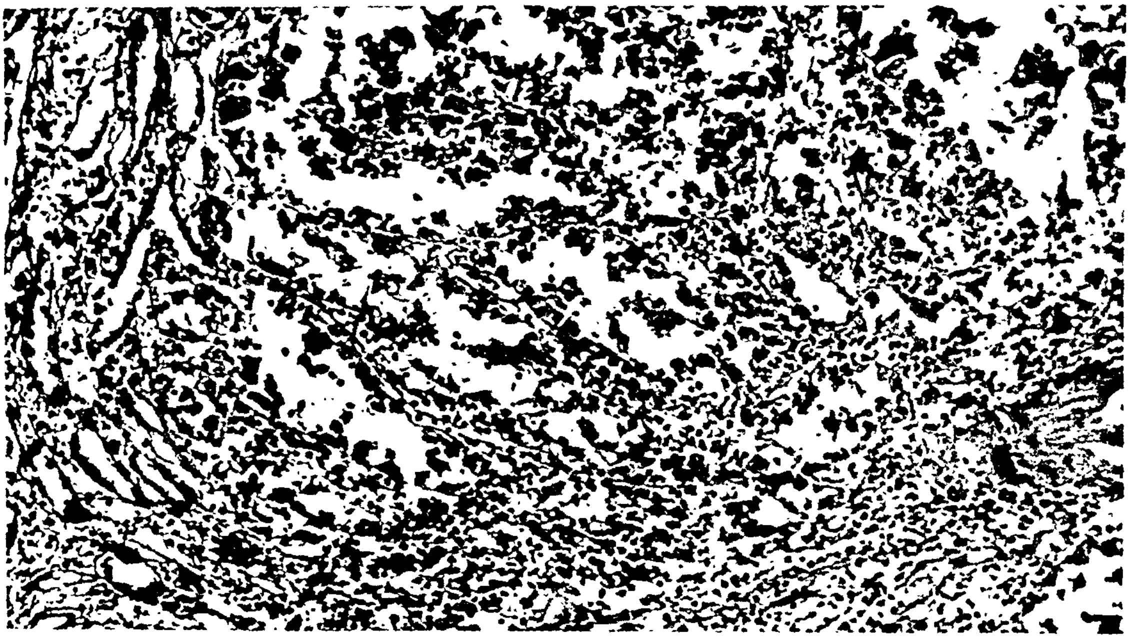

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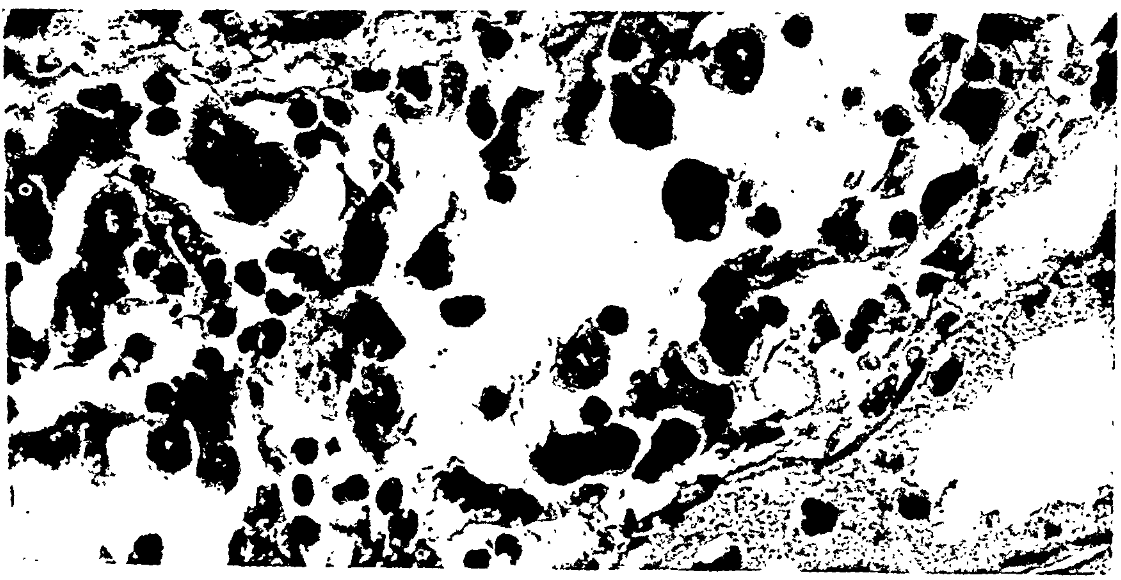

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Lisa

Multicentric Bilateral Renal Carcinoma

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THE PATHOLOGY OF TRENCH FOOT *

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Injuries due to cold have been known for many years, but the study of such lesions always gains new impetus in wartime. In addition to frostbite and trench foot, which have been investigated in the past, new varieties of thermal gangrene in the form of high altitude frostbite of aviators [1, 2] and immersion foot of seamen [3] have made their appearance in the present war. Through the cooperation of a number of medical officers, material from 14 cases (Table I) of trench foot, which occurred in two different theaters of operation, has been received at the Army Institute of Pathology. The alertness of several contributors † who realized the great importance of studying the early phases of the disease process made it possible to obtain tissue from soldiers who died of other causes while suffering from an incipient stage of trench foot. Study of this invaluable material in conjunction with tissue illustrating the intermediate phases and late sequelae has enabled a reasonable picture of the pathogenesis to be reconstructed.

CLINICAL DATA

Almost all of the patients were in their twenties; the oldest was 34. There was one Arab, one Mexican, and one Frenchman; the others were all white Americans from various parts of the Union (Michigan, Maryland, Mississippi, and New York were among the states represented). It is unfortunate that exact data about the weather conditions during the period of exposure are not available in all cases. In some instances the only information submitted was that the patient had "trench foot" or "frostbite"; in others it was stated that the soldiers had been exposed to cold water or to snow.

For one group of patients, cases 8 to 13, all of whom suffered injury during the invasion of Attu, accurate data compiled on nearby ships

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† Lt. Col. Gerson R. Biskind, Lt. Col. Tracy B. Mallory, Major Franklin F. Ferguson, Major David K. Gotwald, Major Harold L. Stewart, Major Edwin T. Thorsness, Capt. George W. Anderson, and Capt. Julius Belinkoff.

and airbases were obtained through the cooperation of the Weather Division, Office, Assistant Chief of Air Staff, Operations, Commitments and Requirements. These records indicate that the freezing point was not reached throughout the week during which exposure took place. The temperature on board ship, presumably within firing range offshore, was 33°F. at its lowest, but usually ranged between 38° and 41°F. The dew point was rarely more than 2 or 3 degrees lower than the temperature, and the relative humidity was often 96 per cent or higher. The wind velocity was less than 10 knots about half the time and lower than 30 knots for most of the remainder of the period. The data recorded at the nearby bases on shore indicate even milder weather. During the first few days of the operation the temperature ranged between 38° and 42°F.; it later fell, 33°F. being the lowest point reached. Humidity was high, but the wind velocity, which was lower than on shipboard, never exceeded 18, and was usually less than 14 miles per hour. The weather conditions on Attu itself were perhaps somewhat more severe in view of the altitude and the mountainous snow-covered terrain, but they never attained so-called "true frostbite" severity. The patients stated that the temperature ranged from slightly above to slightly below freezing. In the coldest areas the ice and snow on the ground melted during the day and froze again at night. Thin skins of ice covered pools and wet areas in the morning. Some of the men's feet became wet during the landing and remained wet for as long as 6 days. The soldiers all kept their boots on for several days.

In 3 cases (nos. 1 to 3), henceforth referred to as "early," material was secured at autopsy from patients who died 7 to 10 days after the onset of exposure, from conditions other than trench foot. Their feet had first become cold and numb, but the pulses remained palpable, and there was no swelling. Edema, increased warmth, and cyanosis appeared soon after rescue and were followed by the development of bullae, hemorrhage, and anesthesia; these lesions were present at the time of death. The blood-tinged fluid from one bulla had a specific gravity of 1.028 and contained 6.8 gm. of protein per 100 cc. and a few cells.

In the remaining 11 "late" cases (nos. 4 to 14) amputation for gangrene was performed 1 to 5 months after exposure. Some patients lost only the toes and others had midtarsal amputations, but most of the operations were low or mid-leg resections. The lesions were usually bilateral and symmetric, but occasionally asymmetric involvement resulted in unilateral amputations.

The process in these late cases had also begun with a "white numb"

TABLE I

Fourteen Cases of Trench Foot

Case	Type of exposure	Duration	Interval from exposure to examination	Type of reaction	Complications
1. Autopsy	"Frostbite"	Not known	7 days	Edema, hemorrhage, cyanosis, bullae	Gunshot wound of head, hemiplegia
2. Autopsy	Cold water	8 hours	10 days	Edema, hemorrhage, cyanosis, bullae	Gunshot wound of leg, fracture, plaster casts
3. Autopsy	Cold and snow	26 hours	10 days	Edema, hemorrhage, cyanosis, bullae	Fracture, gunshot wound of leg, cold injury to hands, hemoglobinuric nephrosis, uremia
4. Amputation	"Trench foot"	Not known	30 days	Gangrene	Infection
5. Amputation	"Trench foot"	4 days	32 days	Gangrene	Infection (*Staphylococcus aureus*)
6. Amputation	Wet and cold	7 days	40 days	Gangrene	None recorded
7. Amputation	"Trench foot"	Not known	42 days	Gangrene	None recorded
8. Amputation	Wet and cold, not freezing	8 days	50 days	Gangrene	Gunshot wound of shoulder, infection (*Staphylococcus aureus; Clostridium welchii*, no gas)
9. Amputation	Wet and cold, not freezing	4 days	50 days	Gangrene	Infection (*Staphylococcus aureus; Streptococcus haemolyticus; Clostridium welchii*, no gas)
10. Amputation	Wet and cold, not freezing	6 days	70 days	Gangrene	Infection (*Clostridium welchii*, gas), cold injury to fingers
11. Amputation	Wet and cold, not freezing	6 days	77 days	Gangrene	Infection (*Streptococcus haemolyticus; Staphylococcus aureus; Bacillus pyocyaneus*)
12. Amputation	Wet and cold, not freezing	Not known	121 days	Gangrene	None recorded
13. Amputation	Wet and cold, not freezing	6 days	143 days	Gangrene	Infection, cold injury to fingers
14. Amputation	"Trench foot"	Not known	Not known	Gangrene	None recorded

stage and evolved through a hyperemic phase into gangrene. At the beginning of exposure the legs were painful for 12 to 24 hours before they became numb or swollen. In one case the feet stayed numb and the soldier "walked on wood" for 6 days, but less than an hour after removal of his boots the feet were swollen and blue, and in 3 or 4 days the toes were already stiff and dry. Although, in one of the early cases, gangrene of the tips of the toes was evident on the seventh day, in most instances 3 or 4 weeks elapsed before frank gangrene set in. In the stage of gangrene the frequent inability to feel the dorsal pedal pulses despite the presence of the posterior tibial pulsations may have been due to local swelling. In the one case in which both the dorsal pedal and the posterior tibial pulses were felt, gangrene extended only to the midtarsal region. The pulses varied in strength from day to day, and there was some improvement after sympathetic block.[4]

As a result of crawling, some patients had lesions on the knees; others had involvement of the hands. Occasionally hyperextension of the toes and a foot deformity resembling Volkmann's contracture developed. *Staphylococcus aureus*, *Streptococcus haemolyticus* and *Clostridium welchii* were among the organisms cultured from secondarily infected lesions; in one case there was inguinal adenopathy.

Cases 8 to 13 were included in Patterson's [4] group. His report gives additional clinical details and includes photographs of some lesions.

Gross Pathologic Findings

The legs in the early cases showed swelling, blebs, bullae, hemorrhage, and cyanosis (Fig. 1). The skin peeled off easily, and the surface was red and injected. The edematous subcutaneous tissues were gelatinous, and the fat appeared abnormal. Usually the damaged portion of the foot was sharply demarcated, at a level slightly below the malleoli, from the proximal normal tissues; moreover, the severely injured superficial layers were also set off from the less involved deep tissues. The subcutaneous veins were dilated, and in one case the dilatation was seen to end abruptly at the demarcation zone. The engorged vascular plexuses showed as a network of tortuous varicosities in the subcutaneous tissues.

In case 2 there were a Pott's fracture about 3 inches above the left ankle, a small penetrating wound on the dorsum of the right foot, and a comminuted fracture of the os calcis which was believed to have interfered with the circulation. Tight casts had also been applied to the legs. A compound comminuted fracture of the lower third of the right femur and penetrating wounds in the posterolateral aspect of the left

thigh complicated the picture in case 3. (A section of the right femoral vein revealed only slight disruption of the wall, scanty cellular infiltration, and a small mural thrombus, while the femoral artery was normal.) In both these cases the possibility must be considered that some of the changes in the tissues resulted from the wounds and fractures, but local damage was minimal in contrast to the extensive injury attributed to cold. In case 1 there were no traumatic lesions of the extremities, but the patient had a severe head wound. Although the effect of the resultant hemiplegia on the peripheral vasomotor disturbances cannot be ignored, the severity of the reaction and the involvement of both feet suggest that the damage to the central system played no significant part in the production of the lesions of the legs. In all 3 cases the lesions ascribed to cold exhibited a basic similarity.

In 3 of the late cases gangrene was restricted to the toes (Fig. 2), but usually it involved the whole foot, almost to the level of the malleoli (Fig. 3). The skin was black and dried, and the tissues appeared desiccated and mummified. In some instances the entire sole, or "sandal area," was blackened, while in others the midportion was spared but the heel was involved. Above the ulcerated zone, 1 to 6 cm. in width, which formed the line of demarcation, the tissues were swollen, cyanotic, and purple-yellow. The ulceration exposed tendons and bone. The soft tissues beneath the black surface membrane were necrotic, and the muscles were either red and putty-like or gray and watery. Although purulent exudate was abundant, foul liquefaction and gas bubbles were present in only one case. The subcutaneous tissues which were not necrotic were gelatinous and edematous, and yellow and orange "congealed" foci were observed in the subcutaneous fat. Thrombosis or thickening of the dorsal pedal, medial plantar, and lateral plantar arteries and other vessels was noted in several instances. Some vessels and nerves frayed out and disintegrated as they passed into the areas of gangrene. Complicating infection resulted in such a variety of inflammatory reactions that it was often difficult to disentangle the lesions due to cold from those produced by the secondary invaders.

Roentgenologic Findings

Roentgenograms of the bones of the foot in an early case and in the 30-day and 70-day cases seemed normal. In one 50-day case both early osseous atrophy and, near some joints, resorption which appeared secondary to infection of the soft tissues were evident. Slight absorption and formation of new bone at the amputated metatarsal stumps and considerable atrophy of disuse were observed in the 143-day case.

Microscopic Findings

Skin and Subcutaneous Tissues

Epidermis. In the early cases there were numerous vacuolated edematous cells in the malpighian layer of the intact skin. Foci of intercellular edema and small vesicles containing fluid and a few leukocytes were noted. Sections through hemorrhagic bullae showed either cleavage in the malpighian layer or stripping off of the basal layer from the papillae. In some places the superficial layers were necrotic. In late cases there was surprisingly little epithelial proliferation at the edges of ulcerated areas. Occasionally the epidermis was thinned out over inflamed and thickened subcutaneous tissue and had lost its pegs, but in other regions it was thickened. In places increased amounts of keratinized material were retained on the surface and in the follicles. In the mummified areas the epidermis was shrunken and desiccated and the clearly defined granular layer stood out prominently. There were bacterial masses on the stratum lucidum, which formed the surface layer. The vacuolation of the cells of the malpighian layer was still evident.

Appendages. The sweat glands in the early cases showed cellular degeneration, and the coils and the ducts were often lined by a fibrinoid layer of homogenized hyalinized cells, pyknotic nuclei, and chromatin débris. The vascular plexus surrounding the glands stood out as a conglomeration of dilated and engorged channels, some of which were thrombosed. A moderate infiltration of leukocytes was present about the glands and vessels. In mummified areas in the late cases the appendages appeared as clumped masses lying in hyaline necrotic collagen. Although the hair follicles were somewhat better preserved, the sebaceous glands were shrunken. In less damaged areas mucinous change in the periglandular connective tissue and perifollicular fibrosis were evident.

Dermis and Subcutaneous Tissue. In early cases the collagen of the dermis was occasionally degenerate or necrotic; the scanty shredded elastica stained poorly. The distal regions showed some of the desiccation so characteristic of the mummified tissue in the late cases. The dermis, in general, was free from cellular exudate, except in the immediate vicinity of congested vessels about the appendages. The stripped papillae, which were shrunken and basophilic, contained nuclear débris and a few leukocytes about the capillaries. Strikingly distended and tortuous papillary loops (Fig. 4) were encountered. Ulcerated surfaces were lined by inflamed granulation tissue or a fibrinoid necrotic membrane. Edematous papillary processes underlay vesiculated epidermis. Agglutinated red cell masses plugged some vessels in the subpapillary

plexus. In the late cases heavy infiltrations of leukocytes, eosinophils, and round cells were evident in areas of cellulitis, but fibrin was not abundant. The collagen was necrotic in areas of gangrene; in the mummified areas the dead fibers, which were shrunken and desiccated, had no nuclei or chromatin débris, were packed together in compact, slightly basophilic bands, and resembled the collagen of burned tissue (Fig. 5). Despite the presence of bacterial colonies in the dead connective tissue, no cellular reaction was evident. Clumped red cell masses filled the dilated blood vessels. Near the zone of demarcation the loose areolar portions of the connective tissue showed edema and mucinous degeneration. In other areas there were dense keloid-like collagenization and scarring, and degenerated elastica (Fig. 6) formed thickened, knotted strands and fragmented segments. In some foci the collagen was broken into irregular granules but stained normally. Cellular infiltration was evident throughout; a few aggregations of cells, which included eosinophils and stem cells, resembled hematopoietic foci, and in one case a marked plasma cell reaction was noted. Increased vascularity, congestion, and hemorrhage characterized many regions, and occasional large collections of hemosiderin-laden macrophages were encountered. Inflamed granulation tissue was the rule in ulcerated areas.

Fat

In early cases infiltration by leukocytes was pronounced in the deeper subcutaneous adipose tissue and in the fat around the appendages even when the overlying layers were not involved. The adventitial cells of the prominent capillaries and smaller vessels in the fat lobules had proliferated. The interlobular fibrous septa showed edema and leukocytic infiltration. There was some fibrinous exudate in the deeper tissues.

Changes in the fat, which were marked in the late cases, were already pronounced in the 32-day case. Foam cells laden with finely divided fat (Figs. 7 and 8) had diffusely infiltrated the fat lobules; for the most part they were smaller than the original fat cells, although an occasional multinucleated form of considerable size was encountered. Rare accumulations of giant cells of the foreign body and Touton types were observed in otherwise unaltered fat. Actual fat necrosis with soap formation was rare; it occurred only in areas of gangrene in the 143-day case. The many minute and few large oil cysts (Fig. 9), which were lined by a layer of foam cells, contained scattered, free, fat globules. Within the adipose cells the fat was occasionally finely divided in place of being smoothly homogeneous. Neither this change in texture nor the previously mentioned lipoid phagocytosis suggested to

me that a reversion to "glandular," or embryonal, fat had taken place, but some other pathologists who studied the sections expressed the belief that such was the case. In a few fat cells doubly refractile sheaves of fine needle-like crystals were noted. Although some changes were especially marked near areas of gangrene and cellulitis, many foci were present in the subcutaneous panniculus, even above the line of demarcation.

Fibrous replacement of adipose tissue took place in two ways. In some regions serous atrophy or actual replacement by loose areolar or mucinous connective tissue (Fig. 10) left a collapsed atrophic structure in which the original outline of the fat lobule was still preserved. In others thickening of the interlobular fibrous septa (Fig. 11) had resulted in conspicuous depletion of the adipose tissue component in the subcutaneous layer.

In many areas of gangrene, especially near mummified regions, the adipose tissue was necrotic; the nuclei had disappeared, but unaltered large fat globules persisted in the dead cells. There was often a faint yellow discoloration, as if there were staining by hemoglobin.

Blood Vessels

Marked engorgement of the vascular tree characterized the early cases. The capillaries and small vessels in the papillary loops, the subpapillary plexus, the networks about the sweat glands and appendages, and the subcutaneous plexus in and about the fat lobules were as clearly outlined as in tissue injected for teaching purposes. Many medium-sized vessels were so dilated, rounded, and thin-walled that they seemed to be paralyzed, as in Ricker's "peristasis." * Extravasated red cells surrounded the engorged plexuses. The erythrocytes in the distended papillary vessels stained poorly, and there was hemolysis in the superficial vascular channels in some regions. In the deeper plexus the red cells were discrete and well preserved, although in some hyperemic foci they appeared spherocytic. Occasionally clumped masses of red cells had become homogenized and hyalinized; other aggregations had been transformed into brightly eosinophilic granular material. Fat, possibly derived from the breakdown of stagnating blood, was present in the lumina of the papillary and subpapillary vessels.

Numerous vessels contained agglutinative erythrocytic thrombi (Fig. 12) of the type which form in stagnant rather than in streaming blood. Only a few pure fibrin plugs were noted, and the amount of fibrin in the other type of thrombus was minimal. In one dorsal pedal

* Ricker, G. Sklerose und Hypertonie der innervierten Arterien. J. Springer, Berlin, 1927.

artery, the wall of which was partially necrotic and infiltrated with leukocytes, there was organization of fibrin at the base of a red cell thrombus. A few small subpapillary channels contained masses of finely granular material (Fig. 13) which presumably were composed of platelets. Platelets were included in the red cell thrombi in medium-sized and large vessels and formed skeletal networks (Fig. 14) both in arteries and in veins. In the veins hemorrhage and thrombosis were often especially marked in relation to the valves. Some vessels contained mixed thrombi composed of red cells, platelets, hyaline material, and enmeshed leukocytes. An occasional yellow-green patch suggested hemolysis. The thrombi usually entirely filled the lumina of the involved vessels, but incomplete plugs were encountered, and rarely mural deposits of hyaline material or fibrin encircled a patent central lumen.

Endothelial damage was not a striking feature, although rare, swollen, vacuolated endothelial cells bulged into the lumina of the smaller vessels. Mural hemorrhage (Fig. 15) and inflammation (Fig. 16) of both plugged and patent vessels, but no periangiitis, were observed. The walls contained leukocytes and chromatin débris, and many coarse and fine granular eosinophilic masses of material replaced the sharply staining cytoplasm of the muscle elements.

Many vessels, especially if thrombosed or inflamed, were dilated, but markedly constricted (Fig. 17) large arteries were also encountered. Vasoconstriction was present even in main trunks well above the line of demarcation, but it is difficult to be certain that such vessels were in the contracted state during life.

In the 32-day case transitions (Fig. 18) from the stage of thrombosis seen in the early cases to the picture of endarteritis obliterans were observed. Proliferation of connective tissue and capillaries and the development of a mucinous stroma were observed in relation to the presumably original thrombi, in which the predominance of red cell and platelet agglutinations and the paucity of fibrin were still evident.

As early as 40 days after the original injury almost all thrombi were already organized, and both arteries and veins showed the features of endangiitis obliterans, even in practically normal tissues above the line of demarcation. The degree of endarteritis varied from slight thickening of the intima to obliteration of the lumen. Slightly involved arteries showed subintimal proliferation of cells, often in a mucinous and edematous matrix (Fig. 19). The intimal thickening was frequently eccentric, but sometimes the entire circumference was involved and in extreme cases marked narrowing of the lumen resulted. The lumina of obliterated arteries (Fig. 20) were filled with fibroblasts, round cells, and hemosiderin-laden phagocytes. Usually the central mass contained

a number of discrete channels which had definite muscular walls and resembled arterioles (Fig. 21). Similar recanalization was noted even in small arteries and arterioles. The proliferative reaction was central to the inner elastic membrane, which was usually intact and not reduplicated. Rarely, involvement of the vessel wall resulted in destruction of the elastica (Fig. 22).

The veins were less regularly involved than the arteries, but they showed nodular intimal thickening caused by edema, mucinous degeneration, and increase in cells, collagen, and elastica. Some were obliterated, but others had labyrinthine lumina (Fig. 23), which may have resulted either from recanalization or from polypoid endophlebitic proliferation. The thickened network of tissue which traversed the central cavity occasionally appeared to be based on pre-existing valvular structures. The strands contained intracellular deposits of hemosiderin and even small, muscled, arteriole-like channels (Fig. 24). Although eccentric involvement occurred, the damage was not confined to the side of the vessel directed toward the skin surface.

Even in late cases occasional vessels which were dilated and thrombosed but still unorganized were found. Some showed necrosis, which was never typically fibrinoid, hemorrhage, and cellular infiltration. Mucinous degeneration, vacuolization, and edema often separated the cells and lamellae of the media even in the absence of intimal change. Perivascular fibrosis, proliferation of adventitial cells, and infiltration of round cells and eosinophils were all encountered, particularly in inflamed regions, but no real periarteritis was evident.

In the areas of gangrene and cellulitis many large vessels and their contents were discernible as ghost structures although they were completely necrotic. A few thrombi were observed; one of these was septic and contained cocci. In mummified areas masses of red cells were still visible in the smaller necrotic vessels, which were lying in the midst of homogenized anuclear necrotic collagen. Some contents suggested fused masses of hemoglobin without cellular structure, but in others the individual red cells were clearly defined.

Muscle

In the early cases the muscle showed degeneration, necrosis, and cellulitis but no atrophy. Although engorgement of vessels, which was so striking in the more superficial tissues, was not observed, thrombi were present in small channels. In case 2 (10 days), in which the leg was in a tight cast for a few days, circumscribed foci of necrosis developed, and an actively phagocytic mononuclear reaction was noted.

Many macrophages had surrounded dead fibers and penetrated the endomysial membrane. Little granulomas, composed of such macrophages, remained where fibers had been destroyed. The infarction observed may have been related to compression since it was absent in the 2 other early cases, but well developed encapsulated infarcts (Fig. 25) were noted in case 5, one of the earliest of the late cases (32 days).

Extensive atrophy (Fig. 26) was noted in the late cases as early as 40 days after exposure (case 6). Although fibrils could be identified, the cytoplasm was usually shrunken and homogenized. Cells laden with yellow pigment lay between the atrophic fibers, which were occasionally separated from the endomysial network by spaces containing edema fluid; the interstitial connective tissue had undergone mucinous degeneration. Proliferation of sarcolemmal nuclei was most evident in the less atrophic areas, but only a few true muscle giant cells were observed. The muscle showed necrosis and inflammation in the areas of gangrene and cellulitis. Circumscribed infarct-like foci of necrosis were present in the zone of demarcation and above. Hyaline degeneration or proliferation of sarcolemmal nuclei was encountered in occasional isolated fibers. Many tendon sheaths exhibited severe exudative and proliferative lesions in which masses of fibrin were more prominent than in the regions of inflammation elsewhere in the soft tissues.

Nerves

In the early cases the nerves which traversed regions of inflammation were swollen and edematous. Even away from such areas degeneration, both of axis cylinders and of myelin, was observed. Large fibers were irregularly broken, beaded, and frayed (Figs. 27 and 28). Myelin balls were distributed along the segmented and fragmented nerve sheaths. Demyelinization was especially marked in the distal portions; in case 1, for example, the medial plantar nerve was extensively damaged (Fig. 29), while the posterior tibial trunk was less involved. Although occasionally swollen Schwann cells contained finely granular fat, the pronounced lipoid phagocytosis so evident in the late cases was lacking. The groups of small and nonmyelinated fibers which presumably represented the sympathetic components of the nerves were not significantly altered either in the main trunks or in the small branches (Fig. 30) near blood vessels; only the large myelinated fibers were affected. The small intraneural vessels showed no essential abnormality.

In the late cases the nerves in regions of gangrene and cellulitis were usually badly damaged, but occasionally a trunk traversing a necrotic area was fairly well preserved. The demyelinization (Fig. 31) which

was seen at all levels was more extensive below the zone of demarcation; often only a few clumps or beaded columns of myelin remained along the axis cylinders (Fig. 32).

Between the nerve fibers there were many foam cells (Fig. 33) which contained sudanophilic material in fine droplets, presumably fat from broken down myelin. In case 13 fine crystalloid spicules also were seen in these lipoid phagocytes. Many axis cylinders had disappeared; those still present were irregular and ballooned (Fig. 34). Damage was usually spotty, so that involved and uninjured fibers were often haphazardly intermingled. Some nerve bundles showed edema and separation of the fibers; actual increase of the endoneurial connective tissue elements may have been present in a few. Perineural fibrosis (Fig. 35) with exaggeration of the epineurium and perineurium was observed, and occasional nerve bundles were partially or completely hyalinized. Many small blood vessels in the nerves were thickened. The degeneration and phagocytosis seen in the subcutaneous adipose tissue were also evident in the epineurial fat.

Bone

Bone was available for study in only one early case. A section of the middle phalanx of a toe revealed no significant abnormality. More extensive study was possible in the late cases. Adjacent to regions of cellulitis were areas of osteomyelitis; the inflammatory exudate had occasionally undermined and eroded articular cartilages. Except in such areas there was little evidence of resorption or of osteoclastic activity; sequestration was not noted. Near the zone of demarcation necrosis of bone had occurred, and the osteocytes had disappeared from the lacunae. A sharply defined layer of viable bone surrounded the dead lamellae. In some regions about the necrotic trabeculae numerous osteoblasts were actively laying down new bone (Fig. 36). In places the bone marrow was necrotic or involved in the osteomyelitic process. Elsewhere it showed serous atrophy, fibrosis, hemorrhage, and infiltration of inflammatory cells. There were many foci of lipoid phagocytosis comparable to those seen in the subcutaneous panniculus; occasional small oil cysts were noted. The altered marrow contained an increased number of thin-walled dilated vessels.

Comment

Skin and Subcutaneous Tissues

Early degeneration of the epidermis after exposure to cold has been repeatedly described,[5-7] but Siegmund[8] expressed the belief that the vacuolation of epithelial cells might be an artifact of thawing. Davis and co-workers[1] attributed the vesiculation and formation of bullae to

transudation. During the regeneration of damaged epithelium, syncytial elements and giant cells may appear, and amitotic division has been said to occur.[5,6] Late atrophy [9] and flattening of the papillae have been noted,[10] and Siegmund also described hyperkeratosis and melanosis of the basal layer. Fuerst reported generalized involvement of the appendages,[6] and Rémy and Therèse [11] observed necrosis, degeneration, and even proliferation of sweat glands. Their reference to hyaline cylinders suggests that they saw the same central layer in the sweat glands which was noted in the early cases of this series. Siegmund mentioned loss of hair follicles and sebaceous glands and the presence of reduced numbers of sweat glands which had swollen basement membranes.

The diffuse sclerosis which may follow cold injury has been attributed to transudation.[9] Siegmund [8] suggested that proliferation of mesenchymal cells and their products resulted from the high protein content of the fluid. Such fibrosis, which Rémy and Therèse [11] first described and termed "lardaceous inflammation," has been held responsible for inelasticity and rigidity of tissues and consequent restriction of movement.[9,12] Marchand[13] expressed the belief that the general thickening of tissues which followed cold injury might have a protective action. Small vessels are constricted by the thickened matrix,[8,9,12] but since telangiectasia also occurs, the resultant picture has many features in common with radiation reaction.[12] Rémy and Therèse emphasized perivascular sclerosis and Siegmund found adventitial proliferation. Hemosiderin-laden macrophages and an increased number of melanophores may be scattered about in the dermis.[8] Degeneration and even disappearance of elastica may occur throughout the connective tissue.

Changes in the subcutaneous fatty panniculus have been described but not stressed, and no special significance has been ascribed to them. Some observers [14,15] made no mention of damage to adipose tissue although it was evident in their published photographs. Siegmund [8] noted free fat droplets and soap formation in the areas of fat necrosis even in early cases; he also found pulmonary fat emboli. In the early cases of the present series the only changes in the subcutaneous panniculus were engorgement of the vascular bed of the lobules and exudation of fluid and leukocytes. Pulmonary fat emboli were observed in two of the three cases in which autopsy was performed, but fractures were present in both instances. Lipoid phagocytosis was not seen within the first month after exposure, and soap formation was observed only in one late case. The fibrosis and atrophy of the subcutaneous fat, which were so extreme in the late cases, have been pointed out by a number of workers.[7,8,10,11,16]

Inflammation of adipose tissue and alteration of fat have been de-

scribed in perniosis.[17-19] The rôle of cold in the production of the adiponecrosis of infants and adults [20] is well known. In a case of "cold allergy" studied by Heid and Fromer,[21] panniculitis developed after brief local application of ice; the process began with leukocytic exudation, and its end-result was a picture indistinguishable from that of Weber-Christian disease.[22] Degeneration of myelin is hardly a specific process, but the possibility that its occurrence in the early cases was a direct effect of cold cannot be ignored. The absence of crystallization and fat necrosis in the early cases is evidence against the theory of a special vulnerability of lipoid tissues to cold, despite Smith's report [23] of fat necrosis and pancreatitis following cryotherapy. Although it has not been determined whether the vascular bed of fat tissue is exceptionally susceptible to damage by cold, it is known that these vessels react to low temperatures. It is probably reasonable to consider that the changes in the fat in the late cases were secondary to the vascular involvement, even though thrombotic and obliterative lesions of vessels were not always demonstrable in regions of injured adipose tissue. Rich [24] suggested that the pressure of clothing on tissue devitalized by vascular obstruction may have led to traumatic fat necrosis.

Nerve and Muscle

Degeneration of nerves after experimental exposure to cold has been described frequently,[5, 7, 11, 25, 26] and the neural changes in frostbite, trench foot, and immersion foot [11, 15, 27, 28] have often been emphasized. Involvement is sometimes evident grossly by the edematous and glassy appearance of the nerves. The earliest changes, consisting of stasis, hemorrhage, cellular infiltration, and exudation of protein-rich plasma, which were described by Siegmund,[8] were not encountered in the present series. Blackwood [14] noted only edema of the nerve-muscle "leash" with separation of the fibers in the one early case of immersion foot which he studied, although he and Russell [26] had found wallerian degeneration early in their experimental material. Degeneration of axis cylinders and myelin, which is characteristic of late stages,[8, 14,] was already present in the early cases of the current group. It has been generally agreed that the neutral fat which was observed in macrophages or Schwann cells is derived from broken-down myelin. Blackwood described regeneration of nerve fibers and proliferation of Schwann cells in late cases. Panchenko [29] and Siegmund [8] noted retrograde degeneration of ganglion cells. Perineural fibrosis and hyalinization of nerves, which have been reported in late cases, have been held responsible for the pain which occurs in some stages of immersion foot [12] and frostbite.[11]

The spotty nature of the involvement in some nerve trunks suggested to Siegmund [8] that the damaged groups might represent sympathetic fibers. In the early cases of the present series unequivocal neural damage was evident, but it was the large medullated rather than the small nonmyelinated fibers which were injured. Blackwood [14, 24] stated that in the nerves which he studied all except the small myelinated and unmyelinated fibers were affected. The destruction of myelin and lipoid phagocytosis were so striking in the late cases of Siegmund's series and of the present group that damage predominantly to medullated fibers seems indicated. Siegmund found only minimal changes in sympathetic ganglia.

Severe degeneration, atrophy, fibrosis, and necrosis of muscle have been described.[11, 12, 27, 30, 31] Although Smith, Ritchie, and Dawson [32] found only slight changes in experimental "trench foot," they stated that severe degeneration and regeneration occurred in "true frostbite." Blackwood [14] expressed the belief that Zenker's degeneration was present in an early case although he was concerned over the possibility that part of the change had occurred post-mortem. Siegmund [8] described waxy degeneration, hyalinization, myolysis, breaking up of the cytoplasm into droplets, anemic necrosis, and gangrene but found no resorptive cells in early cases. The cellular reaction, which was marked in one of the early cases reported here, may have been related to compression by a cast.

In the present series, encapsulated infarcts probably due to vascular occlusion and resembling those described in cases of Volkmann's contracture [33] were present as early as 32 days after exposure (case 5). The muscular degeneration which Blackwood and Russell [26] produced experimentally was of a different type. Although atrophy, which in case 6 was present 40 days after exposure, may have resulted from disuse, Blackwood attributed its occurrence to denervation. Siegmund described both patchy and diffuse atrophy, regeneration, and hyperplasia. Interstitial fibrosis has been considered to follow early edema [8] or rupture of degenerated fibers.[14]

Deformity and rigidity of the foot have been ascribed to damage of the short intrinsic muscles and their nerves with resultant unopposed tensions from the long muscles [4] and to sclerosis of tendon sheaths.[11] The secondary infection and inflammation of synovial surfaces which Rémy and Therèse [11] commented on were also observed in the present series. The frequent development of secondary infection with the usual pyogenic cocci and with other organisms, including those of tetanus and gas gangrene, is well known.[34, 35] The present group was not exceptional in this regard.

Bone

The only significant early changes in osseous tissues were reported by Siegmund,[8] who observed loss of nuclear staining and death of bone. He described the laying down of new bone about necrotic islands and dead lamellae and trabeculae in later cases, a process which was duplicated both in the late cases of the present series and in Ribbert's experimental material.[36] Circulatory disturbances have been held responsible for such osseous changes, which have been compared [8] to those of Sudeck's atrophy. Manteuffel [30] produced thickening of the shaft and widening of the marrow beneath a thinned-out epiphysis in his experiments. Although Brahdy [37] mentioned "dry necrosis," sequestration has seldom been observed.

In late cases osteoporosis may be so marked that the bones cut easily; [8] it can be demonstrated roentgenologically.[38, 39] The widening of the canals in the cortical bone, decrease and thinning of the trabeculae in the spongiosa, and broadening of the marrow spaces account for the rarefaction.[8, 11, 14] Blackwood stated that enough new bone may form to restore the normal roentgenographic appearance. Although productive periostitis and periosteal apposition of new bone have been noted, proliferation of bone is seldom active.[8] Rémy and Therèse described osteophytes at the edges of amputations. Strandell [40] stated that cartilage, and more specifically, the unclosed epiphysis, is especially sensitive to cold, but other workers have not mentioned this point.

In the marrow, edema, serous exudation, cellular infiltration, vacuolation, and myxomatous degeneration may occur.[8, 11, 14] Siegmund described residual bands of perivascular fibrosis in the poorly cellular connective tissue which replaced the gelatinous marrow. The marrow fat in the present series showed the same changes which characterized the adipose tissue in general.

Vascular Changes

The numerous physiologic studies of vascular responses to cold [41, 42] have shown that an initial vasoconstriction is followed by a marked reactive hyperemia. Davis and co-workers discovered by capillaroscopic studies [1] that the normal papillary loops were not demonstrable for as long as 24 hours after high altitude frostbite. Injury by cold is characterized also by an initial stage of spasm, which is succeeded by a hyperemic phase in which the extremities are hot and red,[43] the pulses are palpable,[44] and oscillometric pulsation may even be increased.[28, 45] The occasional conflicting reports of absence or diminution of the pulses [4, 46, 47] may have resulted from observations made either in the anemic vasoconstriction stage or in the later thrombotic phase. Norm-

ally, one reaction to cold is the opening of the arteriovenous anastomosis.[48, 49] Jochim and Hertzman[50] studied the vascular reaction to cold in the finger pad; they found that one group of subjects showed an excessive increase of blood volume before the amplitude of the pulse increased during the phase of reactive hyperemia. They expressed the opinion that arteriolar dilatation, in the absence of adequate opening of the anastomoses, favored the development of tissue damage through engorgement of the subpapillary venous plexus and consequent capillary stasis. Grant[51] was among the first to point out the rôle of the anastomosis in protecting the capillary bed. The histologic appearance of the vascular channels in the current material, especially in the peripheral and mummified portions, is consistent with the view of Jochim and Hertzman. On the other hand, Theis[52] stated that shunting of the blood through the anastomosis resulted in damage through failure of irrigation of the peripheral tissues. He attributed the changes in chronic frostbite to inflammatory involvement of the glomus structures, but his photograph is unconvincing. A satisfactory detailed anatomic study of the arteriovenous anastomoses in lesions produced by cold has not yet been reported. Jochim and Hertzman[53] expressed the belief that the absence of reactive dilatation and venous engorgement in the skin of the forearm resulted from the dearth of anastomoses in this region and suggested that the topography of the lesions in immersion foot might be related to the distribution of arteriovenous shunts.

The importance of transudation of fluid from congested vascular channels, in which permeability is presumably increased, has been stressed by many workers.[3, 54] Going on the theory that the nutrition of the tissues was interfered with by transudation, several[55-57] have suggested therapeutic incision. Smith, Ritchie, and Dawson[58] found that hemorrhage, in addition to exudation, took place especially after warming of the chilled tissues. Davis *et al.*[1] suggested that exudation accounts for the wet forms of thermal injury and that dry gangrene results from thrombosis, which prevents the development of transudation, but Rotnes and Kreyberg[59] attributed the plugging by residual cellular masses to loss of fluid from vessels.

There is no unanimity about the extent and significance of thrombotic occlusions. Thrombosis and organization as the result of different types of exposure and varying degrees of cold, both in human and in animal material, have been described by numerous workers.[7, 11, 16, 28, 30, 31, 60-65] Changes in viscera[66, 67] as well as in extremities have been recorded. Arterial and venous lesions ranging in extent from minor degrees of intimal thickening and endoangiitis to complete

obliteration have also been reported,[1, 16, 27, 28, 30, 39, 60, 61, 68, 69] although such changes have not always been interpreted as the consequences of thrombosis and organization.[15] Leriche and Kunlin [28] stated that they had demonstrated vascular occlusion by roentgenologic methods, but Pässler [45] reported observations to the contrary.

A number of workers [5, 14, 32, 70, 71] have denied the occurrence of thrombi and vascular occlusion after cold injury or have belittled their importance in the genesis of tissue damage in clinical and in experimental material. Blackwood [14] stated that significant vascular changes in the cases of immersion foot which he studied were present only in relation to areas of gangrene and chronic infection and that the minor changes in large arteries were compatible with the age and previous physical activity of the patients. Analysis of the material which he studied may provide a partial explanation of his failure to find lesions comparable to those described in Siegmund's report [8] and in the present paper. The few entire extremities which he received for study were not gangrenous, and in most instances he was limited to examination of gangrenous toes, small portions of tissue, or stumps.

Those who have agreed that thrombosis does occur have differed among themselves as to the particular type of injury by cold in which it appears. Some have insisted [71, 72] that thrombosis takes place only after exposure to "wet cold"; others have stated [73, 74] that it occurs only as a result of injury by "dry cold"; and Talbott [75] claimed that freezing of an extremity was necessary to produce thrombosis. Blackwood,[14] a proponent of the view that thrombi and involvement of vessels are of little significance in the pathogenesis of lesions due to cold, agreed that obliterative endarteritis was a feature of late frostbite.

Greene's experiments [72] with dry cold confirmed a previous observation [50] that clumping of red cells and obstruction of dilated vessels were features of the initial reaction to cold injury. He stated that these clumps were not true thrombi and suggested that the "thrombi" described by earlier workers may have consisted of such cellular masses. Since many of the earlier investigators who described vascular plugs did not include photographs in their reports, evaluation of their observations is difficult, but it is perfectly proper to refer to agglutinations of red cells as thrombi [76] despite the scarcity of fibrin. The amorphous masses of red cells and platelets which were abundant in the early cases of this series resembled both those described by Siegmund [8] and those in the experimental material of Rotnes and Kreyberg [59] and Greene.

The organization, recanalization, and development of obliterative vascular lesions reported in Siegmund's study [34] of thermal lesions among the German troops on the Russian front were duplicated in the

late cases of the present group, although the injuries on the Eastern front were doubtless incurred during freezing weather. He stressed the purely endoangiitic nature of the involvement, even of large arteries well above the zone of demarcation, but admitted that some vascular changes, particularly in areas of gangrene and infection, could have resulted from extension of the inflammatory process. He expressed the belief that mural and intimal thickening might develop after a long period of vasoconstriction. Rémy and Therèse [11] and Siegmund described a train of events leading from hemorrhage and serous exudation to cellular proliferation, formation of mucinous ground substance, elastica, and collagen, and accumulation of phagocytes containing fat and hemosiderin in the vessels.

It seems probable that the vascularized polypoid intravenous vegetations and networks containing hemosiderin, which were stressed by Rémy and Therèse,[11] described in the present paper, and illustrated in other reports,[14, 39] developed from valvular hemorrhages and thrombi. Edwards and Edwards [77] described comparable venous changes in cases of Buerger's disease.

Local hemolysis with staining of tissues and vascular contents has been repeatedly described.[11, 39, 64, 72, 74] Systemic hemolysis, hemoglobinemia, and increased fragility of red cells may also occur under certain conditions.[13, 31, 78] In the experiments of Smith, Ritchie and Dawson, phagocytosis of red cells and leukocytes in regional lymph nodes was noted.[32]

Pathogenesis

It is not clear why necrosis begins despite a presumably adequate if not excessive blood supply. Possibly the tissue damage is a consequence of the poorly controlled blood flow, which, unregulated by the usual vasomotor machinery, is neither paced to the needs of the tissues nor properly routed. The importance of the arteriovenous shunts in this regard has already been discussed. Furthermore, since blood flow in the deep portions of an extremity does not always parallel that in the tissues near the surface,[79] the superficial hyperemia may not accurately reflect the state of the circulation in the muscle. The cramps described by Patterson [4] may result from ischemia involving the nerves as well as the muscles in the deep portions of the limb.

The small arteriovenous differences in oxygen content observed at low temperatures [80, 81] are due to shunting through arteriovenous anastomoses and to diminution in the amount of oxygen released to the tissues. Since chilled tissues require little oxygen, it seems unlikely that simple anoxia is responsible for early tissue damage. Lewis and Love [82] and Lake [9, 54] found that obstructing the circulation to tissues damaged

by cold resulted in less severe injury. Ischemia may act by inhibiting the harmful excessive hyperemia and transudation associated with the reactive vascular response to cold. Fell and Hanselman [83] reported that pressure dressings prevented shock and death after experimental freezing. In the dissenting report of Levin and Khalezkaya [84] it is stated that arterial obstruction and ischemia made experimental frostbite worse. Nevertheless, cooling of the damaged extremity, which reduces both metabolism and blood flow, is an accepted form of therapy.[85] The injurious effect of warming, early sympathectomy, or any measure resulting in excessive vasodilation, is generally known; [86] the idea has been advanced that increased warmth also speeds up the production of harmful metabolites.

The topography of the lesions in trench foot sometimes bears a striking relation to the regions in which there was pressure by footwear.[87] Safford and Nathanson [88] have commented on the development of local necrosis in chilled tissues at points of pressure, but the manner in which compression participates in the production of the lesions is not clear. Lack of active movement, such as the enforced immobility encountered in trench warfare, has been said to result in incomplete emptying of the veins and lymphatics in the already chilled tissues.[89]

It is not known whether the early clumping of red cells represents true agglutination or is merely the result of loss of fluid from the blood. Cold hemagglutination has been described in cases of gangrene of the extremity [90] and Raynaud's disease.[91] Greene [72] found hemolysis a frequent, if not constant, feature in his experiments with frostbite, and the occurrence of local and systemic hemolysis in cold injury has already been mentioned. Consideration should be given to the possibility that released hemoglobin may affect blood vessels directly and that masses of residual stroma from destroyed erythrocytes may block the circulation.

Wieting's report,[61] which includes one of the first pathologic descriptions of trench foot, was entitled "Gefässparalytische Kältegangrän," and the older literature is replete with references to the occurrence of "cold neuritis" in association with frostbite and trench foot. The anesthesia which occurs early as a result of neuritis may lead to "trophic" lesions. The recently developed but cumbersome term "vasoneuropathy" suggests the importance of neural elements in the vasomotor paralysis and the damage to the reflex vasoconstrictor mechanism which follow undue exposure to cold.[3, 9, 92] Study of the present group of cases has failed to reveal involvement of the sympathetic fibers in the main nerve trunks. The medullated fibers, which are said to carry antidromic vasodilator impulses but not vasoconstrictor

stimuli,[93] showed profound alterations, even in the early cases. Blackwood [94] also noted sparing of the small myelinated and unmyelinated fibers. Disturbances of sweating and the sensitivity to adrenalin in cases of immersion foot [43, 95] have been taken as evidence of injury to the sympathetic nerves. Siegmund [8] stated that he was "inclined to believe" in the occurrence of direct damage to the "vegetative terminal reticulum" and the adventitial nerve branches, although he presented no unequivocal anatomic evidence. It is obvious that detailed studies of the sympathetic nerves in injury by cold should be undertaken.

In view of the diffuse damage to all structures in cases of injury by cold, the possibility that low temperature may exercise a direct influence on tissue cannot be overlooked. Despite the existence of temperature gradients [96, 97] between the skin surface and the tissue strata and the insulating action of the fat layer, the deep tissues may become surprisingly cold.[98, 99] Haxthausen [73] stated that cold affects the subcutaneous structures of man more severely than those of experimental animals. Although the mural angiitis in the early cases in this series probably resulted from vascular obstruction, the possibility of a direct effect of cold cannot be ignored. It is known that under certain conditions vessels react directly to low temperature. Angiitis occurs [19, 100, 101] in pernio and chilblains, and a necrotizing arteriolar reaction in perniosis has been observed.[24] However, Siegmund [8] pointed out that the spottiness of the vascular changes spoke against the possibility of a direct effect of cold on vessels. Safford and Nathanson [88] suggested that the damage caused by cold is in reality a "burn" resulting from the sharp rise in temperature which occurs after exposure. Similarities in the lesions caused by burning and by cold obviously exist.[57]

It has been suggested that some persons have a predisposition to injury by cold. It is interesting to speculate about the fact that only a few of the many subjects exposed suffer damage. Some studies [46,102, 103] have apparently indicated the presence of predisposing constitutional elements, but others [104] have disclosed no evidence of such factors. The high incidence of epidermophytosis among soldiers suffering from gangrene after cold [4] brings to mind the now abandoned theory, voiced during the last war by some French authors, that fungus infection was responsible for trench foot.[105] The suggestion that persons who had previously lived in warm climates were more likely to suffer from immersion foot has likewise been abandoned. Jochim and Hertzman [50] noted that some experimental subjects exhibited a vascular response to cold which could be considered abnormal, an observation which doubtless will open up new lines of investigation.

In the early cases of the present series there was no evidence of

antecedent chronic vascular disease, such as arteriosclerosis or endarteritis. Pathologists occasionally encounter cases of gangrene, apparently due to cold, in which it is impossible to decide whether the patient had peripheral vascular disease, such as thromboangiitis obliterans, which was aggravated by frostbite, or whether previous exposure to cold had resulted in vascular sclerosis. Siegmund [8] stated that the late vascular changes in frostbite did not differ from other forms of endangiitis obliterans. The possible relation between injury by cold and Buerger's disease has been mentioned repeatedly.[15, 28, 29, 68, 69, 106, 107] However, other workers,[52, 108] including Buerger,[109] have insisted that many cases of peripheral vascular disease with gangrene had been erroneously considered instances of cold injury. Nevertheless, it has been established that obliterative vascular lesions may follow damage by cold; they may play a rôle in the production of the sensitivity to cold and the disturbance in the neurovascular mechanism which are residua of cold injury. The profound changes in the subcutaneous panniculus described in the present series may also participate in the genesis of the sensitivity to cold.

It has been conventional to differentiate between "true frostbite" and injury produced by chilling under conditions in which the freezing point was not reached. Injury from direct freezing has been attributed to the release of thrombokinase from thawing red cells and subsequent thrombosis [54] and to the release of H-substance from tissue damaged by ice crystals.[110] Rupture of cell membranes, high concentrations of unfrozen salts, desiccation, and disturbances of gel-sol relations have been suggested as means by which tissue is injured.[88] Kochs [111] observed diffusion currents about melting crystals in thawing tissues. Exposure under the weather conditions obtaining after a thaw (Frostschäden ohne Frostwetter) [112] has usually resulted in more injuries than are incurred in actual freezing weather.[113] "Wet cold" is especially damaging because water has a high capacity for absorbing heat (27 times that of air).[98] Injury under such conditions has usually been considered to result from the vascular occlusion which follows chilling rather than from cold directly. Even in "true frostbite," "solidification" may be due to freezing of intercellular fluids without cellular injury,[9] and supercooling [110] may enable tissues to stand extremely low temperatures without freezing. Lake [9] stated that there is often only a small focus of "true frostbite," that the deeper tissues rarely freeze, and that the major part of the tissue reaction, even in cases of true frostbite, may be of the secondary neurovascular type which follows chilling. Other workers [74, 114] have pointed out that frozen tissues are not brittle, and Greene [70] recently voiced the hope that "the theory of solidification

may finally receive decent burial" after having shown experimentally that solidified tissues do not necessarily die. The recent attempt by Davis and co-workers[1] to differentiate between high altitude frostbite and ordinary frostbite was criticized by Safford and Nathanson[88] and by Greene.[70] Until more is learned about the reactions of tissue to low temperature, it may simplify matters to put all injuries caused by cold in one category, since they have comparable clinical and pathologic features.

Although the present study confirms the view that the generalized tissue changes which take place after injury by cold result from the vascular lesions which Siegmund[8] termed "thromboangiopathy" and which Staemmler[115] appropriately described as "anatomic fixation" of the functional disturbances induced by low temperature, a number of points demand study and clarification. There is need for further investigation of the earliest histologic changes which occur immediately after exposure to cold, especially those involving the arteriovenous anastomoses and the sympathetic nerves supplying blood vessels, and closer study of the reactions in adipose tissue. These are only some of the problems which are susceptible to attack by the pathologist; the problems which await the attention of the physiologist and clinician are legion.

Summary

The morphologic changes in 14 recent cases of trench foot have been presented. The conclusion has been reached that all injuries resulting from exposure to low temperatures exhibit a common pattern and result from a similar train of events.

The essential early change is a disturbance in the circulatory mechanism; the consequent stagnation of blood leads to thrombosis and, subsequently, to gangrene, which in many ways resembles ordinary peripheral ischemic necrosis complicated by secondary infection but has certain unusual features. Particular attention has been called to the occurrence of agglutinative thrombosis, profound changes in the fat, and interesting neuromuscular and osseous alterations. The delayed sensitivity to cold which follows apparent recovery may be caused in part by the damage to the subcutaneous panniculus and is certainly related to the occlusive peripheral vascular disease.

Further morphologic studies can contribute to an understanding of the pathogenesis of trench foot. Investigation of the early changes in the myelin sheaths and the fat of the subcutaneous panniculus will determine whether tissues rich in lipoid are specially sensitive to cold. Detailed examination of the sympathetic fibers which supply blood vessels and of the arteriovenous anastomoses will decide whether the initial lesion is vascular or neural.

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DESCRIPTION OF PLATES

Plate 68

Fig. 1. (A.M.M. Neg. No. 82235. Case 3). Hemorrhage, cyanosis, and edema of the foot, 5 days after exposure. The sharp line separating the involved and normal tissues is evident. This appearance is typical of the early changes observed in the present series.

Fig. 2. (A.M.M. Neg. No. 82240. Case 7). Dry gangrene of the toes in a case of severe trench foot, 17 days after exposure. The toes later sloughed.

Fig. 3. (A.M.M. Neg. No. 82238. Case 5). Necrosis of the skin, 12 days after exposure. The blackened, injured tissues are sharply demarcated from the uninjured portion of the limb at the malleoli, the level at which the zone of involvement terminated in most cases.

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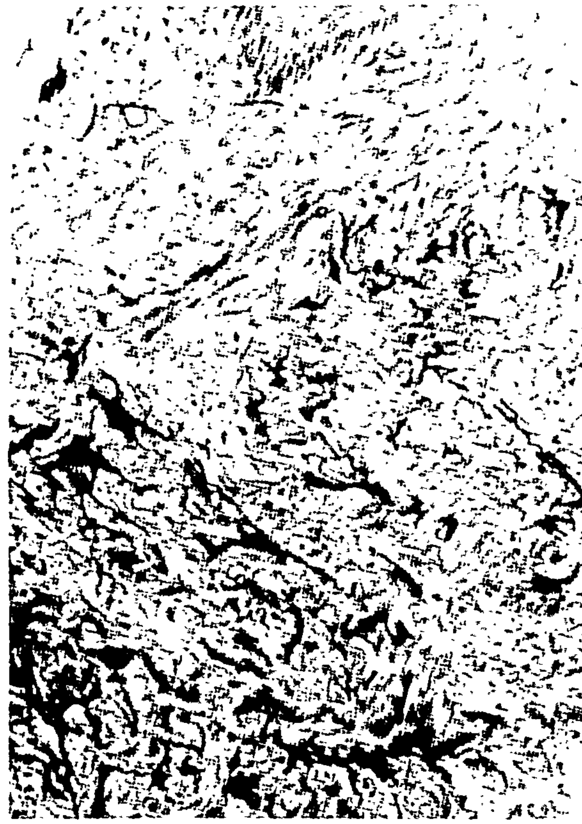
FIG. 4. (A.M.M. Neg. No. 82050. Case 1). Thick section of skin from an early lesion. The papillary vessels are dilated, tortuous, and engorged. Hematoxylin and eosin stain. × 110.

FIG. 5. (A.M.M. Neg. No. 82229. Case 10). Section of skin from a mummified region in a late case. Shrinkage and distortion of the epidermis and subcutaneous collagen are marked. Vascular dilatation and engorgement have persisted; hyalinized and hemolyzed masses of red cells plug some vessels. Hematoxylin and eosin stain. × 110.

FIG. 6. (A.M.M. Neg. No. 81952. Case 9). Degeneration of the subepidermal connective tissue in a late case. The elastica is frayed and fragmented. Weigert's elastica and van Gieson's stains. × 190.

FIG. 7. (A.M.M. Neg. No. 78629. Case 9). Phagocytosis of fat in the subcutaneous adipose tissue in a late case. Foam cells are present throughout the lobule. Hematoxylin and eosin stain. × 145.



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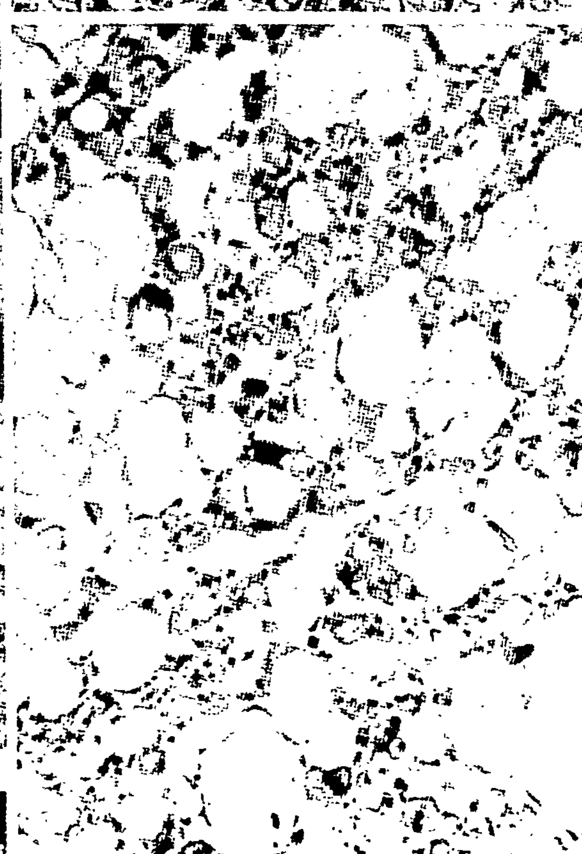

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PLATE 70

FIG. 8. (A.M.M. Neg. No. 81141. Case 9). High-power view of the lipoid phagocytes shown in Figure 7. Masson's trichrome stain. × 1360.

FIG. 9. (A.M.M. Neg. No. 78360. Case 8). An oil cyst, lined by foam cells, in the subcutaneous fat in a late case. Hematoxylin and eosin stain. × 205.

FIG. 10. (A.M.M. Neg. No. 81954. Case 11). Connective tissue elements are replacing the cells of a subcutaneous fat lobule in a late case. Hematoxylin and eosin stain. × 145.

FIG. 11. (A.M.M. Neg. No. 81054. Case 8). Atrophy and inflammation of subcutaneous fat lobules in a late case. The interlobular fibrous septa are thickened. Hematoxylin and eosin stain. × 30.

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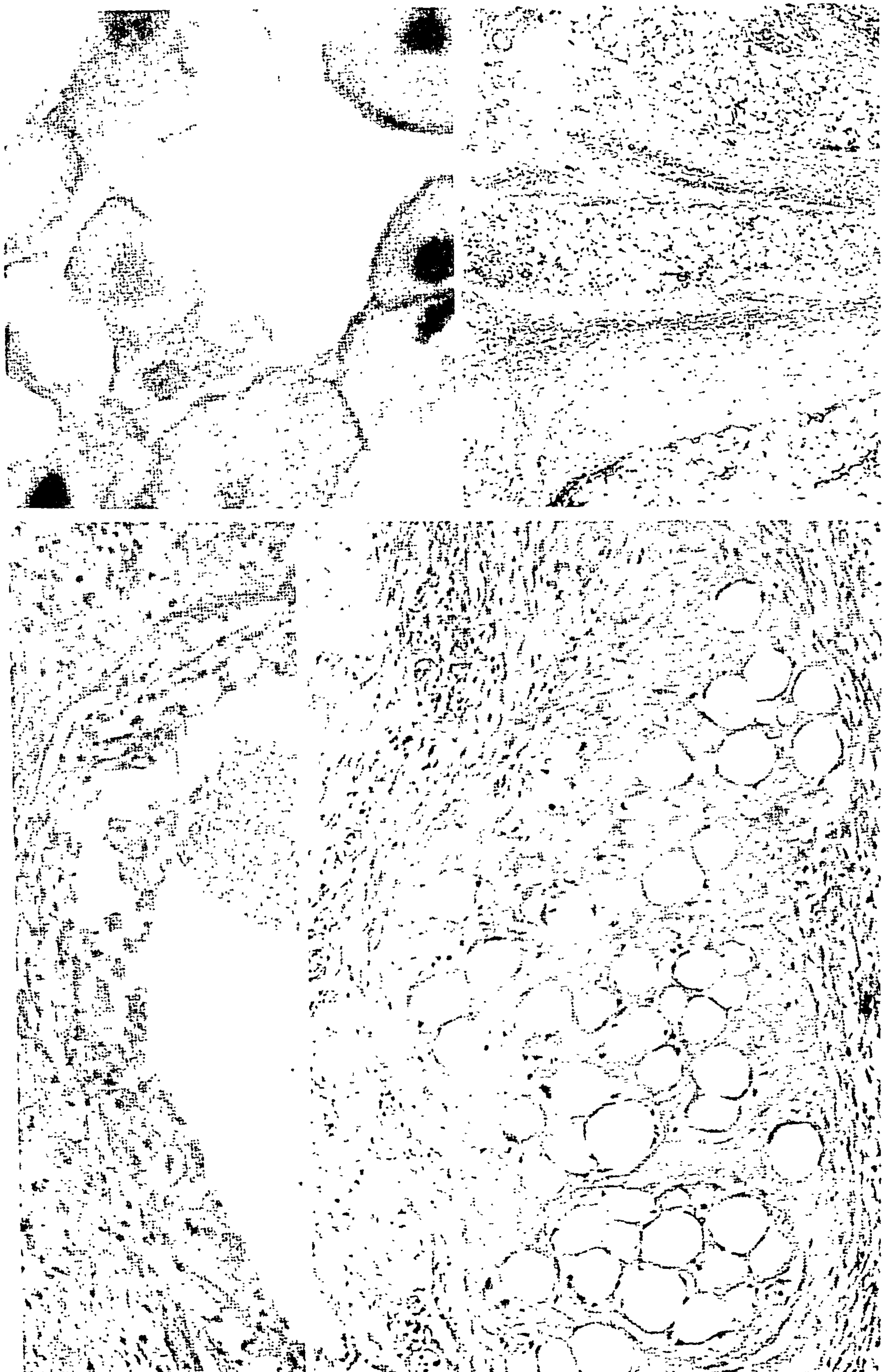

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Plate 71

Fig. 12. (A.M.M. Neg. No. 82059. Case 3). Thrombosis of posterior tibial vein in an early case. The thrombus consists almost entirely of agglutinated red cells. Hematoxylin and eosin stain. × 65.

Fig. 13. (A.M.M. Neg. No. 82054. Case 1). Obstruction of the lumen of a small vessel in the subpapillary plexus by a granular mass of platelets. The section was taken from an early lesion. Hematoxylin and eosin stain. × 500.

Fig. 14. (A.M.M. Neg. No. 82187. Case 1). Thrombosis of the posterior tibial artery in an early case. The thrombus is composed of agglutinated masses of red cells and a framework of platelets. Weigert's elastica and van Gieson's stains. × 32.

Fig. 15. (A.M.M. Neg. No. 81964. Case 5). Thrombosis of an artery from the gangrenous foot in a late case. Organization of the thrombus and hemorrhage into the vessel wall are shown. Hematoxylin and eosin stain. × 50.

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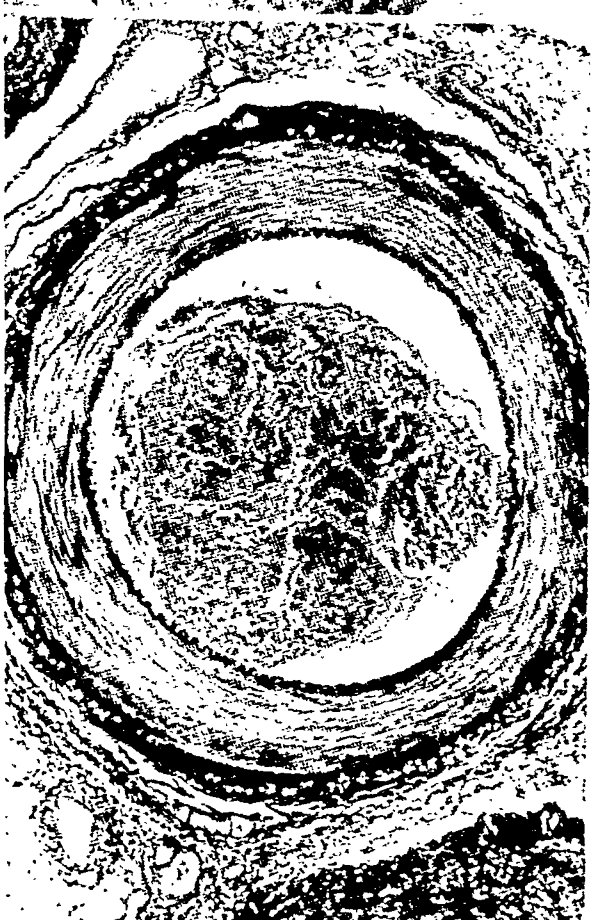

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PLATE 72

FIG. 16. (A.M.M. Neg. No. 82186. Case 1). Mural angiitis in an early case. The wall contains chromatin débris and is infiltrated by leukocytes. The muscular elements are degenerated and the media contains eosinophilic granular material. Hematoxylin and eosin stain. × 500.

FIG. 17. (A.M.M. Neg. No. 82518. Case 3). Constriction of posterior tibial artery in an early case. Weigert's elastica and van Gieson's stains. × 32.

FIG. 18. (A.M.M. Neg. No. 81950. Case 5). Longitudinal section of a plantar artery in the 32-day case. Organization is under way at the head of a thrombus. Hematoxylin and eosin stain. × 75.

FIG. 19. (A.M.M. Neg. No. 78625. Case 8). Proliferation of intima and mucinous degeneration in a small artery. Hematoxylin and eosin stain. × 175.

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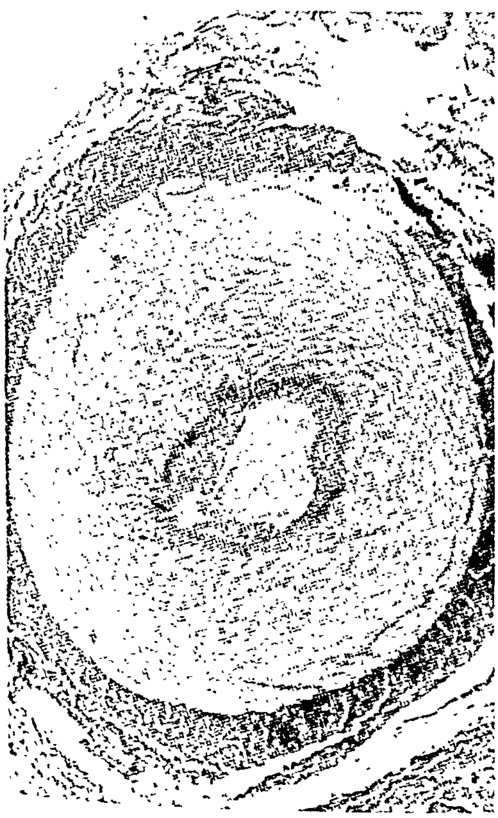

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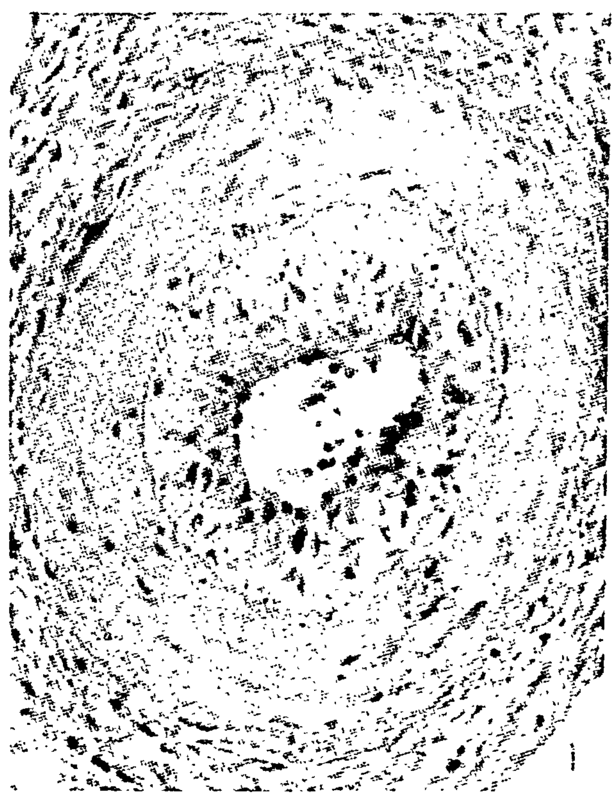

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PLATE 73

FIG. 20. (A.M.M. Neg. No. 78631. Case 8). Obliteration of the lumen of an artery in a late case. The inner elastic membrane is ruptured; the media and adventitia are scarred. Hematoxylin and eosin stain. × 175.

FIG. 21. (A.M.M. Neg. No. 82190. Case 10). Recanalization of a small obliterated artery. Many new channels, some with muscular walls, have formed. Hematoxylin and eosin stain. × 280.

FIG. 22. (A.M.M. Neg. No. 81139. Case 9). Organization and recanalization of an artery in a late case. The elastica is ruptured, frayed and distorted. Weigert's elastica and van Gieson's stains. × 145.

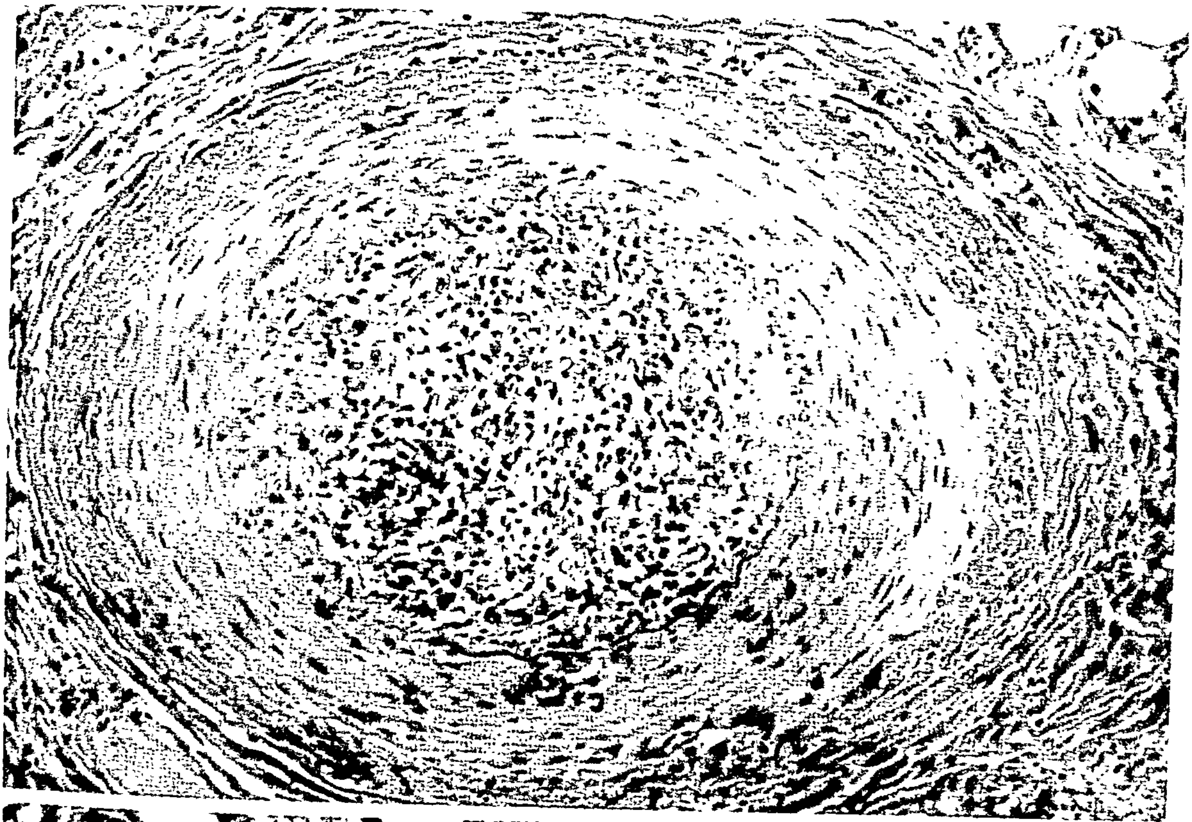

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PLATE 74

FIG. 23. (A.M.M. Neg. No. 81958. Case 10). Phlebosclerosis, intimal proliferation, and labyrinthine recanalization in a late case. Hematoxylin and eosin stain. × 90.

FIG. 24. (A.M.M. Neg. No. 81968. Case 10). Polypoid endophlebitis. New-formed channels with muscular walls traverse the proliferated strands. The dark masses are phagocytes laden with hemosiderin. Hematoxylin and eosin stain. × 145.

FIG. 25. (A.M.M. Neg. No. 81948. Case 5). Necrotic focus in muscle, encapsulated by fibrous tissue and inflammatory elements. Muscle infarcts of this type resemble those seen in instances of Volkmann's contracture. Hematoxylin and eosin stain. × 75.

FIG. 26. (A.M.M. Neg. No. 78633. Case 9). Atrophy of muscle in a late case. The shrunken fibers are widely separated. Hematoxylin and eosin stain. × 145.

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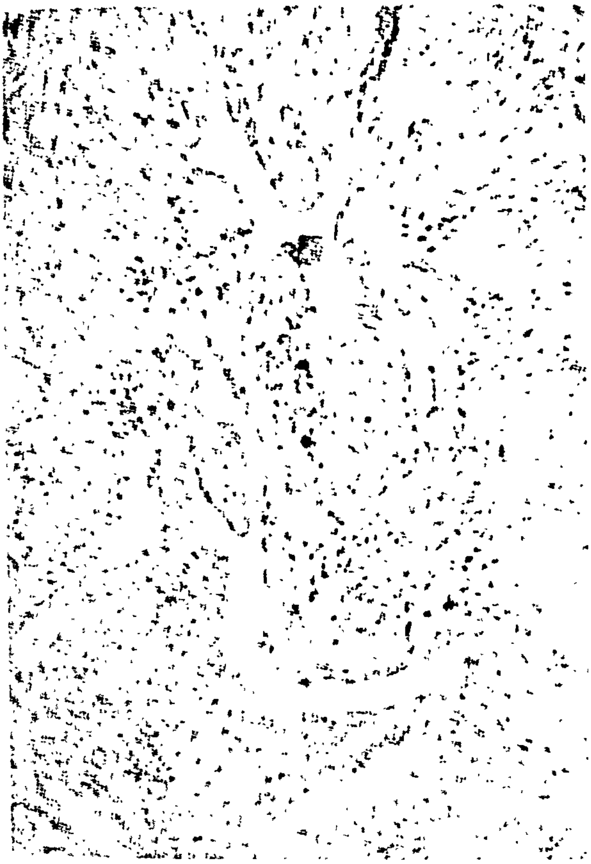

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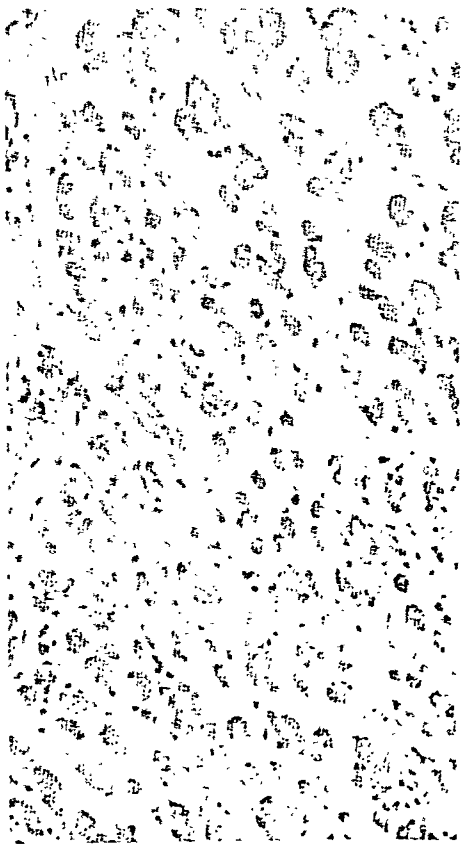

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Plate 75

Fig. 27. (A.M.M. Neg. No. 82864. Case 1). Degeneration of posterior tibial nerve in an early case. The axis cylinders of the large medullated fibers are broken into tortuous segments. Bielschowsky's stain. × 910.

Fig. 28. (A.M.M. Neg. No. 82862. Case 1). Degeneration of the dorsal pedal nerve in an early case. The axis cylinders are fragmented and beaded. Bielschowsky's stain. × 910.

Fig. 29. (A.M.M. Neg. No. 82523. Case 1). Demyelinization of the medial plantar nerve in an early case. A few segments of myelin are all that remain along the fibers. Frozen section. Spielmeyer's stain. × 500.

Fig. 30. (A.M.M. Neg. No. 82531. Case 1). Cross section of portion of the posterior tibial nerve in an early case. The groups of small fibers are undamaged. Bielschowsky's stain. × 500.

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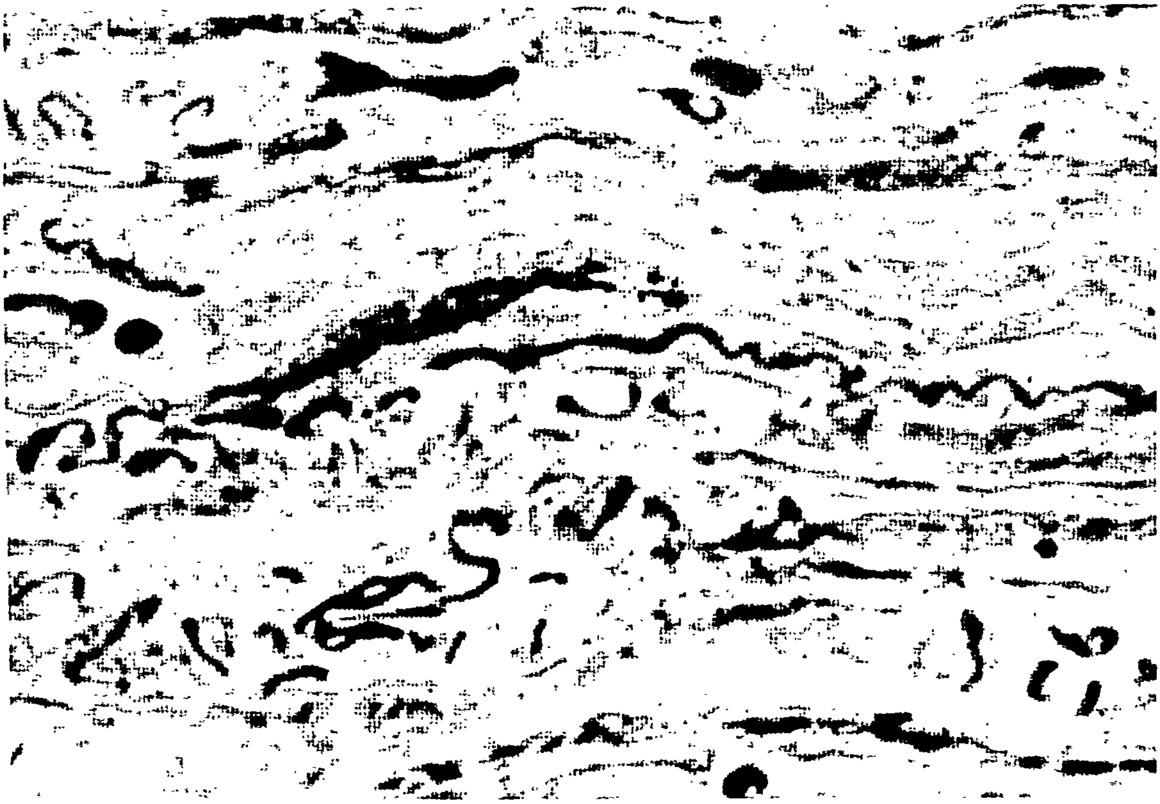

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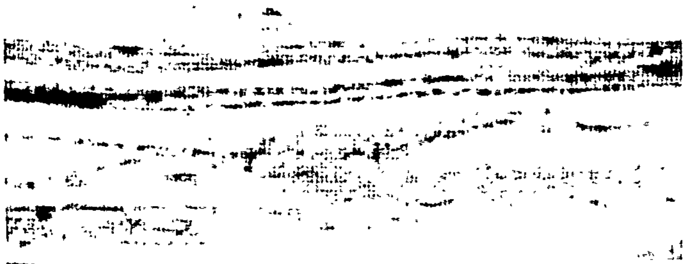

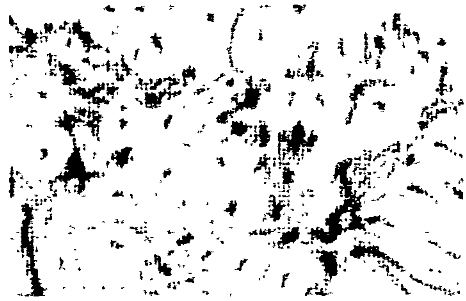
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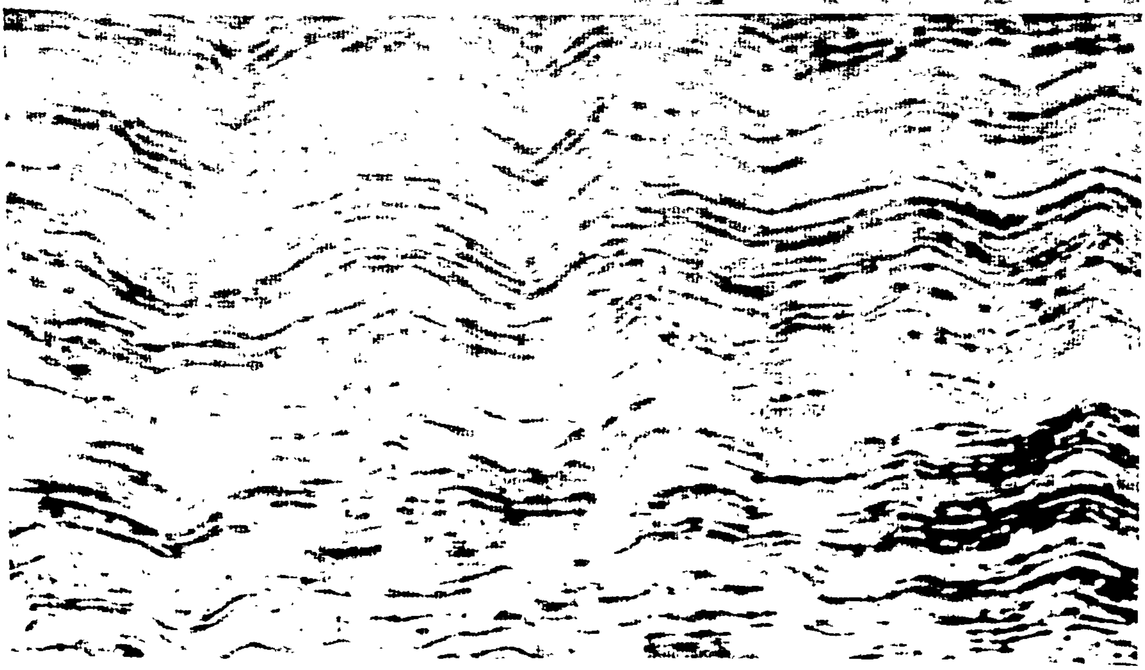

PLATE 76

FIG. 31. (A.M.M. Neg. No. 77251. Case 8). Slight demyelinization of nerve above the region of gangrene in a late case. Frozen section. Spielmeyer's stain. × 125.

FIG. 32. (A.M.M. Neg. No. 77253. Case 8). Marked segmentation, beading, and loss of myelin in a nerve from a region of gangrene. This section is of the same nerve as is illustrated in Figure 31, but it was taken at a different level. Frozen section. Spielmeyer's stain. × 220.

FIG. 33. (A.M.M. Neg. No. 81956. Case 13). Lipoid phagocytosis in a degenerated nerve in a late case. Foam cells are scattered between the damaged fibers. Weigert's stain. × 230.

FIG. 34. (A.M.M. Neg. No. 82524. Case 8). Degeneration of nerve in a late case. Many axis cylinders have been lost and those which remain are ballooned. Bielschowsky's stain. × 550.

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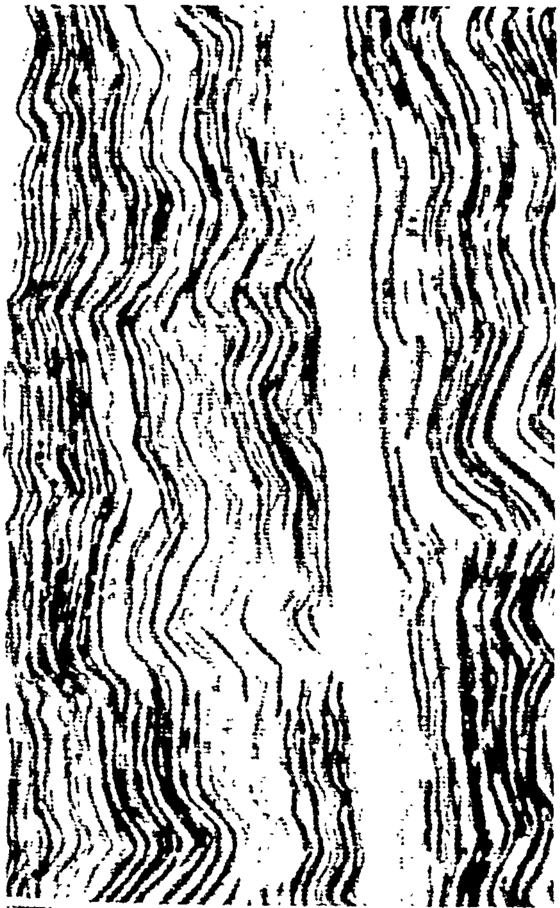

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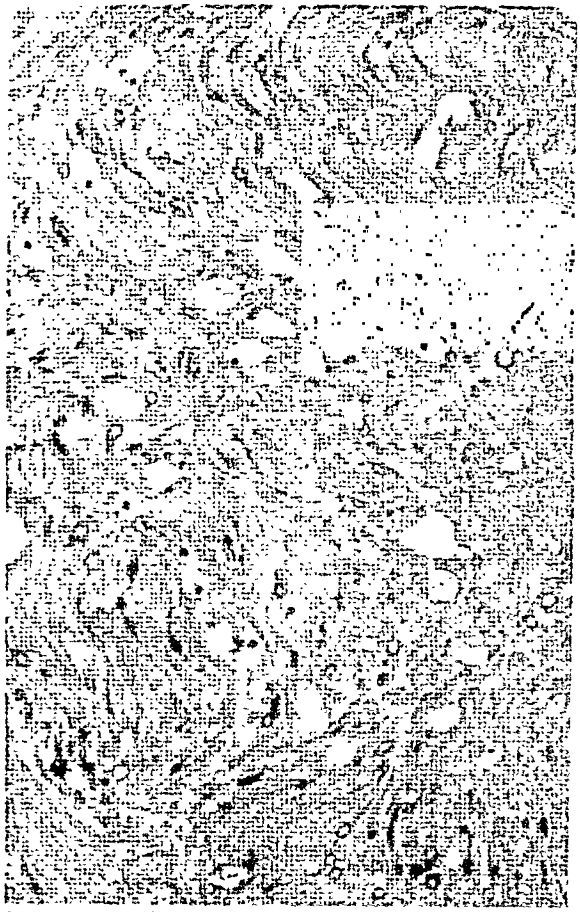

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PLATE 77

FIG. 35. (A.M.M. Neg. No. 81957. Case 11). Sclerosis of nerves in a late case. The nerve bundles are embedded in dense fibrous tissue. Hematoxylin and eosin stain. × 175.

FIG. 36. (A.M.M. Neg. No. 81960. Case 9). Necrosis of bone. Dead trabeculae with empty lacunae have been sheathed by viable bone. Osteoblasts line the newly formed trabeculae. The marrow is fibrotic. Hematoxylin and eosin stain. × 145.

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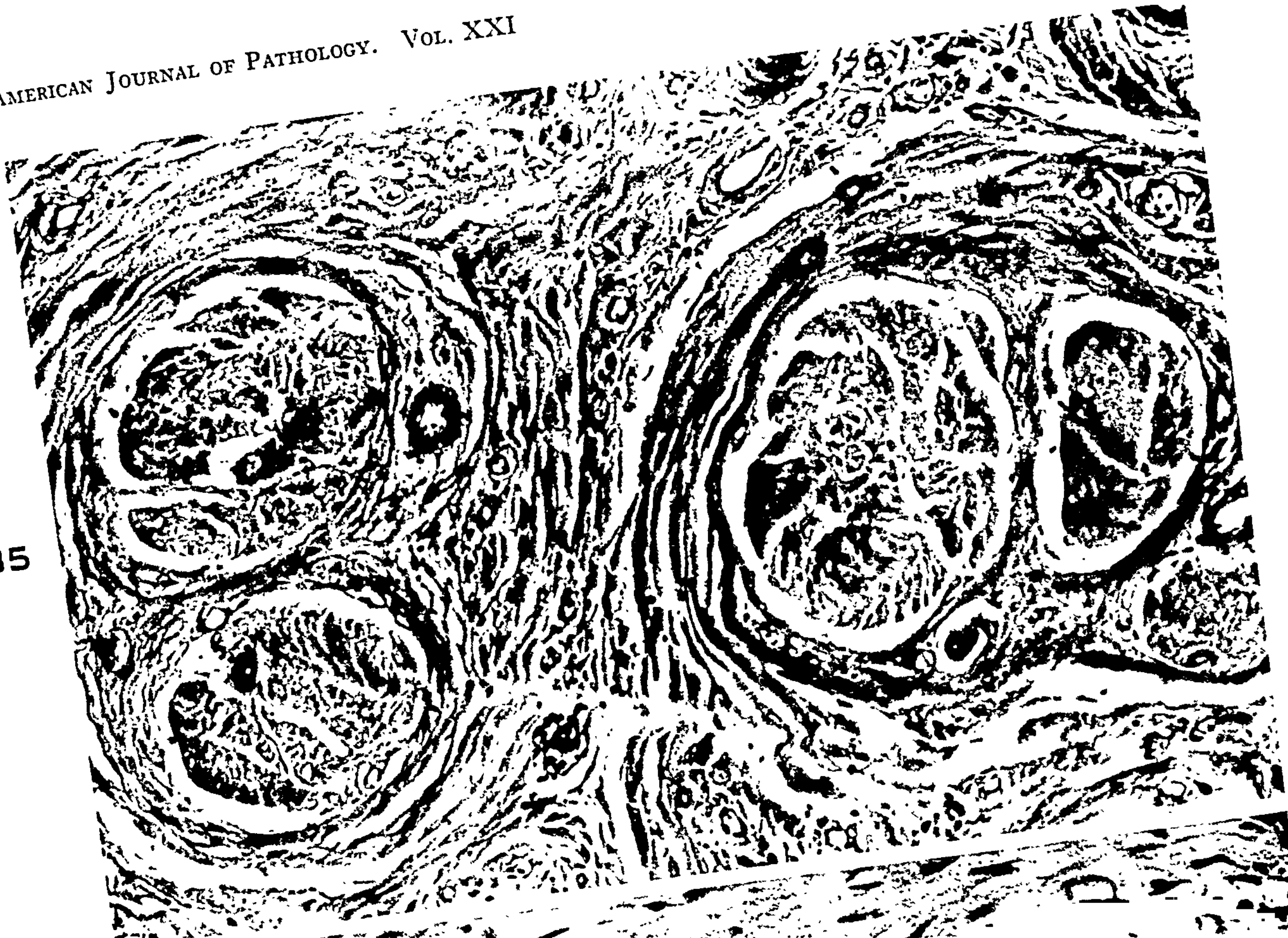

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INTRANUCLEAR INCLUSIONS IN PANLEUKOPENIA OF CATS

A Correlation with the Pathogenesis of the Disease and Comparison with Inclusions of Herpes, B-Virus, Yellow Fever, and Burns *

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(From the Departments of Zoology and Entomology, and Veterinary Pathology, Iowa State College, Ames, Iowa)

The early publications of Lawrence, Syverton, Shaw, and Smith [1] and of Hammon and Enders [2, 3] stimulated us to investigate in more detail the intranuclear inclusions in the mesenteric lymph nodes and in the intestinal epithelium of animals with the disease which they described. This disease has been named spontaneous or infectious feline agranulocytosis,[1, 4] malignant panleukopenia of cats,[3] infectious aleukocytosis of cats,[5] and perhaps we should include also the older clinical designation of feline enteritis.[6, 7] Most of the previous studies made have been devoted to the blood picture. The gross and microscopic appearances have been studied in nearly every case at the height of the disease or some time thereafter and not during developmental stages. The only exceptions are some examinations of bone marrow [1] and lymph nodes.[3] It is our opinion, therefore, that the data to be reported here help to decide which tissues show primary and which secondary reactions, whether red blood cells are involved in either reaction, and also assist in correlating the degree of illness exhibited by the cat with the changes going on within its body, and the extent of gross and histologic lesions with cellular alterations at different stages of the disease. This material permits also a description of the various types of intranuclear inclusions associated with the malady, and of the cycle of ageing which each type follows. When this was done it was possible to compare and classify these inclusions with those produced by other viruses and to evaluate the usefulness of each type for the diagnosis of panleukopenia of cats.

Materials and Methods

The cats used in this work constituted three groups: 2 well cats, 12 post mortem or *in extremis* from the clinic, and 23 receiving the virus under experimental conditions. The well cats were classified as such for comparative purposes in that they did not show symptoms of panleukopenia. Some had intestinal parasites. Animals in the three

* Received for publication, June 19, 1944.

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‡ Now at Small Animal Clinic, 525 17th St., Des Moines, Iowa.

groups represented various breeds and hybrids and usually little or no data about age or previous history of disease were available.

Passage of virus in the preliminary experiments was accomplished by contact of the well animal with one having the disease; keeping them in the same room, but separated, was sometimes sufficient. The virus was passed also by intraperitoneal inoculation of blood or spleen, or spleen and kidney.

Many experiments were failures for one reason or another but the last experimental group gave more usable data than any other and the history of the animals (cats 79 to 83) and conditions of that experiment are presented since these cats will be referred to frequently in the observations which follow. Cats 79, 80 and 81 were litter-mates, said to have been born on April 1, 1943. They were received at the laboratory on October 8, 1943. All were females, all tabbies, and weighed 2425, 2210 and 2445 gm. respectively. Cat 82 was a stray which walked into the laboratory the day the others were received. Its history was not known but it was also a tabby, female, and weighed 1850 gm. The source of the virus used was a spleen received on October 14, 1943, from the Pitman-Moore Co.* by air express, frozen in dry ice. Their cat no. 3835, with a diagnosis of panleukopenia, had been killed on October 6, 1943. Inoculation was made the day the virus was received. The spleen, ground with sterile sand and Locke's solution to make a 20 per cent suspension, was centrifuged for 30 seconds at about 2000 r.p.m. From the supernatant fluid 0.5 cc. was injected intraperitoneally. After each injection a drop or two was cultured on nutrient agar. All cultures remained negative for 4 days, but the culture from cat 80 on the fifth day showed two small colonies. One was identified as *Staphylococcus albus* and the second could not be identified by the tests run, but was not considered to be a common pathogen.†

It was planned to remove tissues from cats killed at intervals of 3 days. At each period the animal was chosen which, on the basis of leukocyte count and general reactions, seemed the sickest.

Most tissues were fixed in Zenker's fluid with 5 per cent acetic acid. For some of the earlier clinical cases 10 per cent formalin was used. The stains employed were hematoxylin and eosin, Giemsa's, phloxine and methylene blue, Masson's trichrome stain, and Feulgen's thymonucleic acid reaction, without or with a counterstain of fast green or orange G. Giemsa's stain was variable in its results which seemed to be influenced by the length of time the tissues had remained in Zenker's

* We are indebted to Dr. W. S. Gochenaur of the Pitman-Moore Co., Indianapolis, Ind., for preparing this material for our use and shipping it to us.

† We are indebted to Dr. R. A. Packer of the Department of Veterinary Hygiene, Iowa State College, who kindly ran the identification tests and gave his findings.

fluid. Phloxine and methylene blue stain was so vigorous in its action that polychromatic effects were obliterated. Delafield's, Harris', or Ehrlich's acid hematoxylin were all satisfactory. The commonly used eosin Y was better than ethyl eosin, in that the latter, like phloxine, was too vigorous in its staining affinities. Feulgen's technic was used to determine whether thymonucleic acid was present in the inclusion bodies and thereby obtain an evaluation of the tinctorial reactions of eosin and Giemsa's stains.

Observations

I. CORRELATION OF CLINICAL RECORD WITH THE GROSS AND HISTOPATHOLOGIC FINDINGS

Some information may be gained by the examination of the internal organs of animals brought to the clinic post mortem and of those killed at late stages in the course of the disease. Frequently they showed hyperemia in the wall of the gastrointestinal tract, in small lymph nodes adjacent to the upper colon, and in the cluster of large and small nodes close to the ileocecal junction. Sometimes there was enlargement of the lymph nodes, but not always. From such animals, however, no information was obtained as to the time when lesions developed.

Among the cats used experimentally, nos. 79 to 82 gave the most exact information on the development of the gross, histologic, and cytologic lesions and their relationship to the animal's behavior, weight, temperature, and total and differential leukocyte counts.

The data are too voluminous to be presented as a single unit and, therefore, have been set forth in Tables I, II, and III and also in the description of the histopathogenesis which follows. Table I gives the clinical data.

The clinical records on the cats were begun 3 days after the animals were brought to the laboratory and were continued for 4 days more before inoculation of virus was made. During this period there was considerable variability in weight and total leukocyte count, which probably represents reactions to confinement in cages in a basement room, to manipulations necessary to obtain the data needed, and to the use of several types of dehydrated food. The weights recorded showed such large fluctuations at first that it was difficult to detect any significant loss until about 6 days after inoculation, with the possible exception of cat 81. Loss of weight occurred about the time the animals refused food and vomiting began.

A significant rise in temperature did not develop until 8 days after

Clinical Data on Cats 79 to 82

Date	Weight in gm. Cat no. 80	82	81	79	Rectal temperature Cat no. 80	82	81	79	Total white blood cells per cmm. Cat no. 80	82	81	79
Oct. 11	2210	1850	2445	2425	102.8	102.4	102.2	103.0	9,700	16,175	17,625	11,250
12	2355	1800	2170	2280	102.4	102.2	103.0	102.8	18,800	14,900	13,600	14,575
13	2330	1810	2175	2355	103.2	102.3	102.5	102.4	21,850	16,000	12,000	13,350
14	2090	1720	2285	2240	103.6	102.6	103.4	103.0	9,800	16,100	12,550	8,800
15	2440	1785	2290	2465	102.4	101.3	102.4	101.3	22,100	12,250	15,200	16,950
16	2245	1745	2345	2360	105.0	104.0	104.4	101.4	5,500	9,250	9,550	15,400
17	2285	1720	2135	2260	104.0	103.0	103.0	102.9	4,550	6,150	5,800	12,200
18		1680	2095	2245		102.4	102.0	102.2		4,550	7,600	3,850
19		1750	2065	2320		102.8	103.4	103.2		5,800	6,000	3,950
20		1700	2025	2225		102.3	101.8	100.0		2,300	6,100	4,000
21			1925	2160			103.0	102.5			3,150	2,250
22			1780	2020			106.3	105.3			200	350
23				1965				103.2				1,450
24				1905				101.0				17,200
25				1830				102.0				25,400
26				1780				102.0				13,100

Differential count of leukocytes

Date	Cat no. 80* Neut.	Lym.	Mon.	Eos.	Cat no. 82† Neut.	Lym.	Mon.	Eos.	Cat no. 81* Neut.	Lym.	Mon.	Eos.	Cat no. 79* Neut.	Lym.	Mon.	Eos.
Oct. 11	45	52	2	1	44	42	3	11	43	52	1	4	52	42	3	3
12	44	55	1	0	40	45	2	13	44	48	1	7	53	46	1	0
13	33	67	0	0	48	37	2	13	42	53	3	2	49	45	2	4
14	39	56	1	4	75	21	1	3	34	62	1	3	38	62	0	2
15	40	52	3	5	48	38	1	13	26	72	1	1	44	50	4	2
16	56	37	1	6	59	29	2	14	86	20	4	8	60	36	0	4
17	68	25	0	7	70	22	0	8	36	60	2	2	88	9	1	2
18					48	45	1	6	48	49	0	3	41	49	8	2
19					69	20	0	11	53	43	4	0	44	54	0	2
20					21	73	0	6	47	50	1	2	22	75	1	2
21									6	90	2	2‡	24	70	6	0‖
22									0	95	0	5§	4	48	8	40¶
23													24	48	12	16#
24													84**	15	0	1
25													89**	10	0	1
26													82**	15	2	1

Inoculation of virus was made after the records were obtained on October 14.
* At necropsy there were found one ascarid and one tapeworm in cat 79, four ascarids and one tapeworm in cat 80 and one ascarid in cat 81.
† Cat 82 revealed coccidial oocysts at fecal examination.
Percentages were based on less than 100 cells: ‡50 cells; § 20 cells; ‖ 27 cells; ¶ 25 cells; # 50 cells.
** All granulocytes were juvenile forms.

inoculation, at which time the leukocyte count was lowest. In cat 79, which survived the crisis, the temperature was normal on the following day.

The drop in total leukocyte count and general trends in the differential count are in close agreement with the reports given in the literature for this disease. The proportion of lymphocytes to neutrophils before inoculation does not agree fully with the averages, and may go beyond the range limits, as given by Ackart, Shaw, and Lawrence,[8] for the normal cat. This may have been due to the new environmental conditions for the animals.

The following descriptions of reactions, when correlated with the data of Tables I to III, give a clear distinction between primary and secondary tissue reactions during the development of the disease and recovery, as well as an evaluation of the necropsy findings.

Cat 80, 3 Days after Inoculation

The behavior, general appearance, and appetite of cat 80, 3 days after inoculation, were normal. All internal organs were normal grossly except the small *lymph nodes* adjacent to the colon. Already there was some hyperemia which gave the appearance of a rosette in several of them. Microscopically, not only the nodes with rosettes but also those which revealed no gross abnormalities were similarly affected. Medullary and cortical sinuses were partially depleted of lymphocytes. More leukocytes were phagocytized in the medullary sinuses than in the corresponding nodes of control cats but this reaction was absent in the cortical sinuses. The erythrocytes were phagocytized in considerable numbers in both cortical and medullary sinuses. They were not disrupted but were ingested intact in as large numbers as it was possible to crowd into the cytoplasm of the macrophage. This was clearly shown after Masson's trichrome stain. The medullary cords were still expanded with lymphocytes. The secondary nodules appeared definitely enlarged but there was no hyalinization such as developed in later stages. The primary nodule appeared about the same as in the normal cats.

Lymph nodules of the terminal ileum showed secondary centers which were not so well defined as in healthy cats, in that the large cells characteristic of this area merged into the primary nodules. No hyalin was present. In the nodules were numerous degenerating eosinophils which were not present in normal cats. Erythrophagocytosis was not evident.

Intranuclear inclusions were present at this time in both the lymph nodes and the lymph nodules of the intestine.

The *colon* showed no changes but the mucosa of the *lower ileum* was affected. No intranuclear inclusions were present but degeneration of the ends of the villi had begun. Beneath the epithelium of the tips a coagulum of serum was conspicuous. The supporting tissue seemed to retract, thus removing the blood supply from the epithelium, and some of the cells already showed autolytic changes in their basal ends. The mucosa of the *duodenum* showed no reaction at this time.

The *spleen* was less reactive than the lymph nodes and nodules. There was extensive disruption, however, of the erythrocytes in the tissue spaces. A few inclusions were present. The only reaction in the *liver* was an increased vacuolization of hepatic cells. Many nuclei in these cells were indented, like red blood cells in hypertonic solution, but were not pyknotic. These changes involved the central

portions of the liver. In the periphery, the sinusoids were almost completely collapsed and the cells and their nuclei were more nearly normal in appearance.

The *pancreas* at this time showed no change.

The *kidney* showed a few tubules which had an acidophilia characteristic of autolysis but the same change and to about the same degree was present in some tubules of normal cats.

Cat 82, 6 Days after Inoculation

Six days after inoculation, cat 82 showed a loss of appetite but the animal's other reactions did not indicate that it was sick.

Macroscopically, congestion had increased in the *lymph nodes* adjacent to the colon and had spread to include those close to the ileocecal junction. All other organs were normal grossly, including the small intestine. Microscopically, the medullary sinuses of the nodes were depleted to about the same extent as they were at 3 days. The cortical sinuses contained not only a great many macrophages loaded with erythrocytes but also many neutrophils. Many erythrocytes within the macrophages were showing degenerative changes. Phagocytosis of leukocytes was greater than at 3 days. Practically all of the medullary cords were collapsed. The primary nodules were unchanged but the secondary centers contained cells with strongly eosinophilic cytoplasm and after Masson's trichrome stain there were seen droplets of hyaline material among them.

The primary *nodules of the terminal ileum* were not conspicuously changed although they contained more cells with large nuclei than is normal. The eosinophilia and hyalinization of the secondary nodules was similar to that found in the lymph nodes. As in cat 80, no phagocytosis of erythrocytes or leukocytes was found, but there was a conspicuous infiltration of eosinophils, in many of which the granules were coalescing and degenerating.

Intranuclear inclusions were present in both lymph nodes and nodules, and were slightly more abundant at this time (Table III).

The *colon* showed no significant microscopic changes. The *lower ileum* was severely damaged, in that the villi had been reduced to about half their normal length. The mucous cells were depleted and disorganization was beginning in the epithelium of the glandular area. The *duodenum* still lagged behind in extent of tissue destruction although intranuclear inclusions were as numerous here as in the lower ileum.

In the *spleen,* red blood cells did not fill the tissue spaces so abundantly as was found at 3 days; the splenic pulp seemed more closely packed.

All of the nuclei of the *liver* had a spherical shape, the cytoplasm was more vacuolated. Sinusoids and hepatic cords were intact.

In the acini of the *pancreas* the cells were intensely stained and their basal ends sometimes shrank away from the cell wall when fixed.

The *kidney* showed a greater number of tubules with cells with vacuolated cytoplasm and showing autolytic changes than were found in the healthy cats or in cat 80.

Cat 81, 8 Days after Inoculation (Height of the Disease)

Cat 81 was killed at the end of the eighth day, instead of the ninth as originally planned, because it probably would not have survived the night. It was too weak to stand. Hyperemia was widespread in the lymph nodes along the colon and ileum. The walls of the small intestine showed extensive areas of congestion except in the region of the duodenum. The same reaction was visible also in the spleen and in the uterine tubes. Fat was yellowish and not so abundant as in cats 80 and 82. Other visceral organs appeared normal grossly. Microscopically, the cortical and

medullary sinuses of the *lymph nodes* adjacent to the colon were fairly well filled with cells, more so than they were earlier in the disease. Those in the lymph nodes adjacent to the ileocecal junction were entirely depleted so that the meshwork of reticulocytes was clearly shown. Phagocytosis of erythrocytes was still abundant in a node by the colon but was less in an ileocecal node. In both, the picture of phagocytosis had changed, in that very few erythrocytes within the macrophages were intact but had disintegrated and the cytoplasm of the macrophages had frayed out in many cases. The blood plasm contained much cell débris. This reaction at the terminal stage of the disease was noted by Hammon and Enders.[2] The medullary cords showed varying degrees of collapse and expansion. The centers of all nodules were strongly hyaline but some showed early stages of regression. Few cells were contained within the hyaline area. The large cells characteristic of the normal secondary nodule lay at the periphery of the hyaline mass and beyond them the character of the primary nodule was essentially the same as before.

The hyaline areas in the *lymph nodules in the terminal ileum* showed early stages in regression. Beyond the centers there was a more or less uniform mingling of large and small cell types. There was no phagocytosis of blood cells. Degenerating eosinophils were present but less abundant than in cats 80 and 82. The intranuclear inclusions had decreased to about the number found at 3 days.

The *colon* even at this stage showed little reaction to the disease beyond some loss of mucus from the goblet cells at the base of the glands and greater loss of epithelial cells. This agrees with the observations of Lawrence *et al.*,[1] but the damage to the *lower ileum* was even more extensive than before, in that the villi were reduced to short knobs covered with low columnar epithelium. Many cells were lost from Lieberkühn's crypts, and those remaining had compensated by changing to a squamous shape. Mucous cells were practically gone. The *duodenum* for the first time showed extensive destruction of villi, including the epithelium of Lieberkühn's crypts. Fewer intranuclear inclusions were present in the duodenum than in cat 82 but in the lower ileum they were about as numerous as they were 3 days earlier.

The hepatic cells of the *liver* now showed no vacuoles and the cytoplasm was densely granular. Much of the cytoplasm was torn away, leaving frayed cells with nuclei at the margin. The nucleoli were small. The sinusoids were filled with coagulum and there was slight perivascular infiltration around some vessels.

The shrinkage of acinar cells in the *pancreas* was greater than in cat 80.

The *kidney* showed a large number of damaged tubules, represented by cytoplasmic vacuolization in some cases and by autolytic eosinophilia in others.

Cat 79, 12 Days after Inoculation (3 Days after the Crisis)

Twelve days after inoculation, cat 79 showed increased alertness and activity over that shown when the leukocyte count was lowest. However, the animal still did not eat and there was continued loss of weight. It is doubtful whether it would have recovered ultimately. Grossly, neither lymph nodes nor small intestine showed hyperemia. The other internal organs appeared normal except that the gallbladder was greatly distended with bile. This was not due to blockage of the duct since it was possible to milk the bile into the duodenum with the handle of the scalpel. The cortical and medullary sinuses of the *mesenteric lymph nodes* were filled with cells and closely resembled the normal condition, both as to cell types and distribution. Only a few erythrocytes were ingested and the stages were all late in the process. No ingested leukocytes were seen. There was wide variation in the filling of the medullary cords, ranging from empty to full. The nodules of the node had changed markedly. All hyalin was gone and it had been replaced by aggregations of small lymphocytes which were surrounded by larger lymphocytes having the general

appearance of cell types found in a normal secondary center. These, in turn, were surrounded by a mixture of cell types characteristic of the cortex.

Hyalin had gone from some of the *lymph nodules of the terminal ileum* and was about half regressed in others. The stellate appearance of the hyalin suggested that regression occurs from the periphery by the invasion of small lymphocytes. Cells peripheral to the small lymphocytes showed a uniform mingling of large and small types. No phagocytosis of erythrocytes or leukocytes was seen and there were no eosinophils.

The *colon* was still unchanged. The mucosa of the lower *ileum* showed improvement over that found at the crisis of the disease. Mucous cells were more numerous. The villi were still lower but better covered with epithelium. That these changes represent improvement and not a less critical illness is indicated by the regression of hyalin in the lymph nodules of this tissue. The *duodenum* showed little or no improvement except that the epithelium covering the villi was more nearly normal than in cat 81.

In the *spleen,* hyaline areas in the nodules had almost entirely regressed. Débris of previously ruptured erythrocytes was still present. One giant cell was seen, but no intranuclear inclusions.

The *liver* also showed a little improvement, in that some vacuolization of the cytoplasm of the hepatic cells had returned. In the sinusoids were coagulum, neutrophils and other reacting cells.

The *pancreas* revealed a greater disruption of acinar organization than was found at the crisis of the disease. There was leukocytic invasion.

The *kidney* also showed some recovery from the condition found at the crisis, in that the tissue disorganization was less and the striated cuticular borders were more definite.

Clinic Cats

In the group of 12 cats from the clinic, the medullary and cortical sinuses of the *mesenteric lymph nodes* varied from those filled with phagocytic macrophages to those which had a mixture of phagocytes and normal lymphoid cells. The medullary cords varied from collapsed to full. Hyaline centers were usually present in the nodules but in many examples they showed varying degrees of regression and in some they had gone completely. The primary nodules were not particularly reactive, except in one case where there was marked loss of cells; in others the organization of the primary nodule was more normal than in cat 79. Intranuclear inclusions were found in the lymph node in one clinic case.

Practically all of the clinic cats showed greater disorganization of the *lymph nodules in the terminal ileum* than did the experimental cats. The lymphoid cells were diffusely scattered and all traces of organization into primary and secondary nodules were gone. Some showed areas of necrosis and neutrophilic infiltration, not found in the experimental cats. A few clinic cats were less severely injured in that the nodules showed hyaline centers. Eosinophils were observed in only one case.

The mucosa of the *colon* in 4 clinic cats was half eroded but post-mortem changes may have played a large part in this. The material collected in the clinic has been of little value in studying the destruction due to disease in the intestinal tract because post-mortem changes were also present. In the *lower ileum* the villi were sometimes mere stubs of connective tissue without a trace of epithelium covering them. In most cases, practically all of the glandular epithelium was destroyed also. Material from the *duodenum* was not taken from these animals.

The *spleen* showed varying degrees of hyalinization; in some it was conspicuous and in others it had entirely regressed. Inclusions were present in one case.

The *liver* damage was in nearly every case more severe in the clinic animals than in the experimental ones. In some, the hepatic cords became dissociated into isolated cells, and the sinusoids were disrupted and often collapsed. The cytoplasm was

dense and darkly staining. In some livers there was nuclear hypertrophy with aggregations of oxychromatin around the nucleolus somewhat similar to Figure 1. In one clinic cat, stages of recovery had set in: infiltration of phagocytic cells, decreased staining of cytoplasm, and a tendency to form numerous basichromatic granules in the nuclei. There were numerous focal areas of reorganization. In one of these there was found an intranuclear inclusion of the homogeneous type.

The *pancreas* was taken in only one animal. There was extensive acinar disorganization accompanied by leukocytic infiltration.

The *kidneys,* like the other tissues from clinic animals, showed a great variability. None were more nearly normal than cat 81 and most of them revealed disorganization with degeneration of the tubule cells.

Certain conclusions seem warranted on the basis of the histologic changes during the development of the disease. The lymphoid tissue is the first to react. There is a draining off of available lymphoid cells from the stroma of the lymph nodes, later followed by depletion of the cords, but the nodules of the cortex remain intact throughout the disease. The nodules react by changes in organization, develop hyalin in the center, but quickly return to normal after the crisis has passed.

The phagocytosis of erythrocytes is a reaction which begins early in the disease. The ingested cells in the early phases are normal in appearance. The reaction is still strong at the sixth day although disintegration of previously ingested material is apparent. By the time the crisis is reached there is no longer ingestion of fresh erythrocytes, only the completion of the process of disintegration. These facts may indicate, possibly, that the virus is disseminated by adherence to the surface of the red blood cells, or simply that early in the disease there has been hemorrhage into the lymph channels. Macchiavello and Bezerra Coutinho [9] noted that the blood was the first tissue to lose its infectivity.

Since this disease depletes the circulatory leukocytes so severely, it might be expected that there would be severe phagocytosis of these cells. However, there is no marked phagocytic action against the leukocytes in the early phases of the disease. It reaches a peak about the sixth day, but at the time of the crisis and in the recovery period the leukocytes ingested are few or none. The number of leukocytes in proportion to the erythrocytes is so small and the reaction against the erythrocytes is so vigorous that significant phagocytosis of the leukocytes may have been obscured. It has been suggested that they may be lost due to a leukotoxic substance and by way of the intestinal lumen.[10] Degeneration of the eosinophils was found only in the lymph nodules of the lower ileum. These cells may be associated with parasitism and may have no relationship to the action of the virus. On the other hand, there seemed to be a cycle of eosinophilic reaction in the three cats which had the same parasites, and the cells are usually absent in cats seen at the clinic.

The mucosa of the ileum is the second tissue to react and thence the reaction progresses to the duodenum. The stomach and esophagus were not examined.

The reaction in the spleen is limited mostly to the lymphoid areas. The liver, pancreas, and kidney are the last visceral tissues to respond to the development of the virus and they show their greatest damage when the disease is at its height or even after the crisis has passed.

The destructive action on the liver may be even greater than in the lymph nodes but there is nothing to indicate that it is the primary focus of action for the virus. In view of the extensive liver damage, it is interesting that Kikuth, Gönnert, and Schweickert tried a vitamin C diet in the treatment of this disease. They obtained negative results.[5] Apparent success of vitamin C as a clinical therapeutic measure in combination with sucrose has been experienced by Riser.[11]

The reactions of the animal do not become those of a "sick cat" until late in the disease when the macroscopic changes of the internal organs are far advanced. Thus, in dealing with the disease clinically, it is possible that a cat may not be brought to the attention of a veterinarian until the blood count has returned to normal. In that case, at the time of death, diagnostic gross, histologic, and cytologic criteria have disappeared, except for the clue which may come from the presence of juvenile forms in the circulating blood.

II. CELLULAR ALTERATIONS

The intranuclear inclusions described when the agent of the disease was first identified as a virus have been the principal subject of this investigation. It has been determined how many different types occur, their relative number at different phases of the disease, their distribution in the various tissues, the cycle through which they pass from young to old stages, and finally a comparison with other known inclusions.

Two types of inclusions have been discovered in this material, granular and homogeneous. The granular type is further divisible into diffuse granular and clustered granular.

Clustered Granular Intranuclear Inclusions

The clustered granular intranuclear inclusion is the most commonly found type and is reported by Hammon and Enders,[2] by Lawrence *et al.*,[1,*] and by Kikuth, Gönnert, and Schweickert.[5] In its fully de-

* A comparison of inclusions in our material with that reported at the University of Rochester Medical School was possible through the courtesy of Dr. Jerome T. Syverton who sent us six stained slides.

veloped form it is morphologically identical with inclusions of yellow fever as described by Cowdry and Kitchen [12] and on the basis of structure should be classified in the same group as yellow fever. However, in the cycle of changes which involve early and late phases, it is readily distinguishable from the inclusions of yellow fever and under certain conditions may resemble those of herpes.

As with all studies on the cycle of inclusion formation, the early stages are difficult to find and to distinguish from the nuclear variations common to any reacting tissue. The most reliable criterion that a nucleus is developing toward inclusion formation is the pulling away of the normal reticulum, which is usually somewhat basophilic, toward the nuclear membrane. This reaction in itself is not distinctive, in that it occurs frequently in nuclei under various abnormal conditions, but if an inclusion is developing, strands of linin network lag behind on which there may be a few oxychromatic granules at the interstices (Fig. 2). These granules are lightly staining, are colored only by the acid dyes and are Feulgen negative. Sometimes they are not distinguishable as discrete granules but appear to be a lightly colored, homogeneous mass (Fig. 3). This may resemble a plasmosome of an amphinucleolus in that basichromatic granules are sometimes associated with it, but the color reaction in our preparations is that characteristic of an inclusion and not a plasmosome.* Even in these acidophilic masses a suggestion of granular structure may be seen, if an optical system of maximum efficiency is employed (see footnote, page 457).

These early stages differ somewhat from those found by Cowdry and Kitchen [12] for yellow fever. In yellow fever the first granules to appear have already separated from acidophilic linin fibers and are located in the center of the halo, but in panleukopenia of cats no early stage was found having only a few discrete granules which were lying free from linin fibers in the center of a halo, although a thorough search was made for them. Additional evidence that in this disease the earliest granules are associated with an acidophilic linin network is derived from a study of the diffuse granular inclusion to be described later.

Frequently, nuclei are found in which there are accumulations of discrete granules clustered near the amphinucleolus (Fig. 1). This

* Usually, plasmosomes observed in this material (if a stain giving a good range of tinctorial effects is used) are neutrophilic (Figs. 1, 3, and 14). The term "neutrophilic" is used here to denote a gray color which is due to the mixing of acidophilic and basophilic substances in the same structure. The gray color is a result of the blue of hematoxylin mingled with the orange of eosin. The same result occurs when the red of Feulgen's stain is blended with fast green as a counterstain. In an earlier paper an inclusion showing a similar reaction was called a "dark body" or an acidobasophilic inclusion.[11]

might be suggestive of an early stage except that the generalized reaction toward margination is lacking. Moreover, it is a type of reaction frequently found in nuclei under abnormal stimulation when no inclusion bodies are developed.

Once the early stage has passed, the subsequent stages are clearly distinctive. The granules become more numerous and are of uniform size, about one-third to one-half of a micron. The staining reaction is still eosinophilic with no basophilia (Fig. 4). A few delicate, radiating strands extend from the inclusion to the nuclear membrane. Concomitant with the development of the inclusion body is a completion of margination except for the nucleolus (Figs. 4 and 5). The nucleolus lags behind and is sometimes stretched across the nucleus as a darkly staining band, as shown by Kikuth, Gönnert, and Schweickert.[5] The lagging of the nucleolus is characteristic of yellow fever also and was used by Cowdry and Kitchen [12] as a distinguishing feature between that disease and herpes.

A fully formed inclusion may show a clear orange color with eosin but more often its tinctorial reaction changes toward a superimposed basophilia. The granules become more numerous and closely packed (Fig. 5). The radiating fibers may be few or none. It is suspected that they are present even when invisible because the inclusion tends to have irregularities in its contour which are associated with radiating linin fibers when these can be detected.

When basophilia * has developed in the inclusion body, it is regarded as having passed its ascendency and is now entering its terminal stages. The inclusion becomes more compact, basophilia increases, and the nucleolus tends to marginate at this time if it has not already done so.

Ultimately the inclusion becomes homogeneous with a well developed halo and very few, if any, radiating strands (Figs. 6 and 7). It is impossible to see granules in the homogeneous stage but they can be distinguished in all of the phases which lead up to it. Morphologically, it resembles an isolated plasmosome but it is usually distinguishable by the staining reaction in that it has a mauve color after hematoxylin and eosin, whereas the plasmosome is usually neutrophilic. In Figure 7 is shown both an isolated plasmosome and an inclusion body. The cell shown in Figure 14 has a typical isolated plasmosome. Not only is the tinctorial reaction of the particular cell shown in Figure 14 distinctive

* The term "basophilia" will be used to mean that a slight amount of basophilic substance is present so that the color reaction of the eosin has changed from a coral pink to a mauve or magenta. The term has not been used to mean that a structure has taken on the same staining reactions as chromatin.

enough to separate it from a late granular inclusion, but a late inclusion would not be found associated with such an early phase of nuclear margination.

Diffuse Granular Inclusion

The diffuse granular intranuclear inclusion is less common than the clustered granular inclusion. The ratio may vary from 1:5 to 1:50 (Tables II and III). Since at least several hundred inclusions should be examined critically in order to arrive at any opinion concerning their course of development, it has been a handicap that diffuse granular inclusions were relatively so few. However, their morphologic configuration is sufficiently definite in the fully formed condition to classify them as a distinct type. Margination of chromatin is more vigorous in the early phases of this type than in the clustered granular type but during the margination there remains behind a more extensive and more conspicuous acidophilic reticulum. On this reticulum, at the interstices, are scattered eosinophilic granules. They have a tinctorial reaction identical with the reticulum and are not refractile so that it is sometimes difficult to see the granules in the early stages (Figs. 8 and 9). Figure 8 is quite similar to Figure 2 but the following criteria were used to distinguish them. In the early clustered type the linin fibers radiating from the granules may be slightly basophilic whereas in the diffuse type they are clearly eosinophilic throughout. In the former the granules are more discrete and independent of the reticulum and in the latter they are hardly more than enlargements at the crossing of the fibers.

As the diffuse granular inclusion develops, the acidophilic reticulum increases in amount and so do the granules, but they remain located at the interstices as in the early stages and still do not develop refractile properties (Figs. 9 and 10) as do the clustered inclusions.

The chromatin and nucleolus react differently in the two types also, in that complete margination comes relatively earlier in the diffuse granular type and the nucleolus never remains in the center of the nucleus. In most cases it has disappeared entirely by the time the inclusion is fully formed.

The inclusion, therefore, is composed of granules plus reticulum and the reticulum has many radiating strands which extend to the nuclear membrane. As a result, the halo is less clearly defined than in the clustered type. In some examples the reticulum fills nearly all of the nucleus.

The late stages are subject to the same limitations and doubts as are the early stages because the examples are so few. The inclusions develop a superimposed basophilia and the linin network contracts

somewhat (Fig. 11). Since the granules still remain attached to the reticulum, the contraction of the network produces small vacuolar spaces. As contraction and increased basophilia continue, a stage is reached comparable to the homogeneous phases of the clustered granular inclusion except that the vacuolization is retained (Fig. 12). Further evidence that such an inclusion is derived from a diffuse type is given by the large number of radiating fibers in contrast to the small amount of reticulum and few radiating fibers of the clustered type.

No morphologic or developmental differences whatever have been found between the diffuse and clustered granular inclusions which appear in the lymph glands and those in the intestinal epithelium. Nor do the clustered granular inclusions develop in one cell type and the diffuse inclusions in another. Since they are found together and in similar cells, the difference in reaction is probably a physiologic one. This idea is supported by the fact that sometimes a young, diffuse inclusion may have at one side a group of granules characteristic of the clustered type (Fig. 13). In spite of the fact that structures characteristic of both types may occasionally be present in the same nucleus, yet it is our opinion that one is not a developmental stage of the other but that they represent two variations in nuclear reaction to the disease stimulus and that each inclusion type follows its own cycle of ageing.

The time involved in the inclusion cycle could not be learned from the data available in this study, except that the inclusion develops and disappears quickly and in this respect is similar to the monocytic reaction in the virus of the submaxillary gland of guinea-pigs.[13]

Homogeneous Inclusions

In this material the fully formed homogeneous inclusion, not of the type derived from a granular inclusion but from a plasmosome, as will be shown later, is usually neutrophilic with eosin Y (Figs. 14 and 25), but may be stained red with ethyl eosin or phloxine. It lies in the center of a halo, has few and usually no radiating strands, and its edge may or may not be slightly refractile. The chromatin may be partially marginated, as in Figures 14 and 25, or completely marginated, as in Figures 15, 16, and 26.

The developmental stages were most clearly demonstrated in the liver of cat 82. The inclusion is derived from a plasmosome portion of an amphinucleolus. In the cell type from which it develops (Fig. 23) the plasmosome is surrounded by basichromatin granules. The reticulum is normal and granules are scattered between the nuclear membrane and the nucleolus. Practically every nucleus in the liver has this

structure, but occasionally in sections of liver there are groups of cells in which marginating chromatin may be found leaving the plasmosome (Fig. 24). In Figure 25 the process has progressed far enough so that the plasmosome would probably be called a homogeneous intranuclear inclusion and would be classed as Cowdry's type B.[14] Complete margination was not found in the liver cells but was observed in the lymphoid tissue and intestinal epithelium (Figs. 15, 16, and 26). A similar derivation of an inclusion from a plasmosome was described by Birch and Lucas [15] as a response to subcutaneous injection of aluminum oxide in guinea-pigs, except that in that case the plasmosome gave an acidophilic reaction. In the cat, panleukopenia may produce the same acidophilic reaction in some cells (Fig. 15) although the usual reaction has been neutrophilic. Figure 15 appears almost identical with an early stage in the development of intranuclear inclusions by the virus of the submaxillary gland of guinea-pigs in which homogeneous eosinophilic and neutrophilic inclusions were in the same nucleus (Fig. 5 in paper by Rosenbusch and Lucas [13]). It was concluded by Rosenbusch and Lucas that the difference in staining probably represented difference in age, the younger being more acidophilic and the older more neutrophilic. The same conclusion seems valid for reactions such as shown in Figure 15. Sometimes a mixture of materials may be found in which basophilic masses are mixed with acidophilic substance (Fig. 16), or they may be so intimately mingled (Fig. 17) that it is impossible to determine whether the inclusion was derived by way of a plasmosome or a granular inclusion.

Other peculiar reactions of the plasmosome have occasionally been observed. One of these is an exaggeration of vacuolization. Vacuolization in itself is a common reaction but only occasionally does it go as far as shown in Figure 26.

Even in the prophase of mitosis, a plasmosome can look like a typical homogeneous inclusion (Fig. 29-b). Upon superficial examination the plasmosome inclusion (in this case, double) seems to be surrounded by a halo and the chromatin to be marginated. The chromatin is actually marginated but it is assembled in the form of a spireme, as may be seen by examining the nuclear sphere at all levels of focus (Fig. 29-a). By a study of earlier prophases, it was determined that isolation of the plasmosome and margination was the normal procedure in mitosis in this material (Figs. 27 and 28). In Figure 27 the chromatin makes many contacts with the plasmosome, as it does in the resting stage, but as the spireme thread is organized, it gradually pulls away from the plasmosome (Figs. 28 and 29).

Mitosis and Intranuclear Inclusions

Mitosis in cells which contain cytoplasmic inclusions of virus origin is well known.[16, 17] Ivanovics and Hyde[18] described lobulation and amitosis associated with virus III in tissue culture but no instance of true mitosis has been recorded in cells which bore a granular intranuclear inclusion.*

The intestinal epithelium is particularly favorable material for study in that the rate of mitosis is higher than in most tissues of the body. Although diligent search was made among many hundreds of inclusion-bearing cells, only two examples were found (Figs. 18 and 19). Figure 18 is a prophase spireme and shows a clustered granular inclusion in about the middle phase of development. The spireme threads are fairly well organized at the left side of the nucleus but poorly so on the right side. Figure 19 is not a normal mitosis and perhaps represents a late prophase or an abortive metaphase. It is doubtful that mitosis would be successfully completed. It would be interesting to know, because of its bearing on cytogenetic problems, whether the failure is due to mechanical causes or to deprivation of oxychromatin.

Cellular Death and the Inclusion Body

It has been maintained in previous publications[13, 15, 19] that the development of an inclusion body does not in itself predestine the death of the cell. Thus, death, when it comes to inclusion-bearing cells, is a result of the environmental stimuli which are set up by the action of a virus or other foreign agent. This conclusion is borne out in the present study, in that lethal changes may be superimposed at any phase of the development of an inclusion body. In Figure 20, necrosis and shrinkage of the nucleus have come at a time when the inclusion is at a phase of development comparable to that shown in Figures 4 and 5. In Figures 21 and 22 that change has come at later phases. Nuclear death has progressed farther in Figure 22 than in Figure 21 in that karyorrhexis has begun, as evidenced by the isolated chromatin granules and invisibility of nuclear membrane. In the lower cell of Figure 22 the chromatin has liquefied. This cell may have had an inclusion body, judging by the position of the plasmosome, but there are no other criteria to determine the point.

* Since this was written, two reports of mitosis in nuclei which contained inclusions have come to our attention. One concerned a homogenous type associated with thyroid tumors (Stewart, C. F. Intranuclear inclusion bodies in carcinoma of the thyroid gland. *Am. J. Cancer*, 1939, 37, 196–200). It would be interesting to know whether these inclusion bodies had origin from plasmosomes. The second describes a peculiar homogenous body found in renal nuclei following administration of bismuth (Pappenheimer, A. M., and Maechling, E. H. Inclusions in renal epithelial cells following the use of certain bismuth preparations. *Am. J. Path.*, 1934, 10, 577–588).

TABLE II

Mitosis and Inclusions in the Epithelium of the Lower Ileum

Cat no.	Days after inoculation	Time killed	Total cells counted	Mitotic figures		Clustered granular inclusions		Diffuse granular inclusions		Ratio of diffuse to clustered inclusions	Empty nuclei	
				No.	Per cent	No.	Per cent	No.	Per cent		No.	Per cent
77	Normal cat	2:30 p.m.	10,000	111	1.11	0	—	0	—	—	0	—
78	Normal cat	4:00 p.m.	10,000	99	0.99	0	—	0	—	—	0	—
33	In extremis	11:00 a.m.	10,026	49	0.49	661	6.59	77	0.77	1:8.6	13	0.13
80	3 days	8:30 p.m.	10,024	116	1.16	0	—	0	—	—	0	—
82	6 days	2:45 p.m.	7,081	102	1.44	175	2.47	8	0.11	1:22.4	1	0.014
81	8 days (crisis)	10:00 p.m.	2,725	31	1.14	81	2.97	9	0.33	1:9.0	2	0.073
79	12 days	12:15 p.m.	1,0030	90	0.90	2	0.02	0	—	—	0	—

Relative Number of Inclusion-bearing Cells and Mitoses during the Course of the Disease

It was the original purpose of this investigation to determine to what extent the inclusion bodies could be used in clinical diagnosis. It was soon discovered that they had less value than originally hoped because at the death of the animal they sometimes were present but usually were absent. The cause for this variability was discovered when cats were killed during development and recovery stages of the disease.

Counts were made on the number of mitotic figures, the number of diffuse and clustered granular inclusions, and isolated plasmosomes. As to plasmosomes, only those were included which resembled the ones shown in Figures 14, 15, 16, and 26, and not those which arose as part of the mitotic process (Figs. 28 and 29). The counts are summarized in Tables II and III.

Inclusions first appeared in the lymphoid tissue, not only in the nodes adjacent to the colon where the first congestion of blood vessels was observed, but also in the lymphoid nodules of the lower ileum. By the sixth day after inoculation, up to the time of lowest leukocyte count, the inclusions were present in both lymphoid tissue and intestinal epithelium. They might be found throughout the small intestine, including the duodenum. They were absent from liver, pancreas, kidney, and adrenal during the full course of the disease but a few were present in the spleen. In the

lymph nodes the greatest number of inclusions were found on the sixth day and in the intestinal epithelium the greatest number were present at the height of the disease. This agrees also with observations on the histopathology of the disease. Three days after the crisis had passed the inclusions had disappeared. Only two were found in numerous sections of lymph nodes and intestine examined. Thus, it is evident that the inclusions disappeared soon after the crisis was passed. Support for this idea comes from the examination of clinic cats; only 1 of the 12 showed any inclusions. It is for this reason that it is claimed that negative findings at necropsy have relatively little value in the determination of the cause of death. On the other hand, a positive finding is highly specific.

TABLE III

Occurrence of Inclusions in the Lymph Nodes Adjacent to the Colon

Cat. no.	Hypertrophied cells counted	Clustered granular inclusions		Diffuse granular inclusions		Ratio, diffuse to clustered	Isolated plasmosomes		Empty nuclei	
		No.	Per cent	No.	Per cent		No.	Per cent	No.	Per cent
80	1005	49	4.88	1	0.099	1:49	0	—	0	—
82	1000	60	6.0	4	0.40	1:15.0	2	0.20	6	0.60
81	917	28	3.05	6	0.65	1:4.7	0	—	0	—
79	1000	0	—	0	—	—	0	—	0	—

Empty or ghost nuclei (Fig. 30) were recorded in the counts because they are abundant in yellow fever material and following severe burns. In panleukopenia of cats they are almost completely absent and none of them show the enormous hypertrophy described by Cowdry and Kitchen [12] for yellow fever.

Plasmosome nucleoli which are free from all basichromatin and lie in a halo are also rare and have little significance except to indicate that a nucleus may react in other ways than by the development of an inclusion body.

It was anticipated that the extensive destruction of epithelium during the development of the disease and its rapid repair afterward would be reflected in a change in mitotic rate. Counts of mitoses were made in the region of the glands and did not include the villi. It was discovered that the mitotic rate is remarkably constant and the values obtained differ but little from those found in the normal cats. Since periodicity of mitosis has been reported in several publications,[20-23] it was unfortunate that the animals were not killed at the same hour in the day. It should be emphasized that casual examination of the slide may give an erroneous impression. Before the counts were made, it seemed cer-

tain that cat 79 had many more mitotic figures in the field than the others.

Cat 33 was included in Table II because it had more inclusions than any other animal studied. The data are relatively meager. It was one of the earliest cats used experimentally and was destroyed in a moribund condition 3 days after intraperitoneal injection of 2 cc. of blood from a diseased cat. When killed it had a leukocyte count of 1800 per cmm. and a temperature of 103.0° F. The cat may have been infected spontaneously sometime before the inoculation was given.

Comparison of Inclusions of Panleukopenia of Cats with Those of Herpes, B-Virus, Yellow Fever, Severe Burns, and with Other Granular Types

If studies on the morphology of intranuclear inclusions are going to be of practical value to the pathologist in his diagnosis of disease, it is necessary that distinguishing differences be set forth clearly. Cowdry and Kitchen [12] observed that the nucleolus is not so readily marginated in yellow fever as in herpes simplex and that there is a slight basophilia of herpes inclusions which is absent in those of yellow fever.

The developmental stages of the inclusions in herpes and B-virus,* as well as some additional facts not reported in the literature on the morphology of later phases, are given since they also are valuable in distinguishing inclusion types. The earliest stage which can be identified is not granular but is represented by one or more homogeneous or amorphous masses which are not refractile. The masses are acidophilic. At the time of their appearance the chromatin may not be completely marginated (Fig. 31). There is no difference between these masses and those which frequently appear in nuclei under stimulation as droplets of oxychromatin. In fact, the two are probably identical. Later, there develops, in association with these masses, uniformly fine acidophilic granules (Fig. 32 and lower left cell of Fig. 34). It has been impossible thus far to determine whether the granules arise within the homogeneous masses or invade the cell, as has been maintained by Nicolau and Kopciowska.[24] As the granules increase in number they become evenly spaced and it is possible to distinguish a delicate reticulum joining them (Figs. 33 and 34). This reticulum has not been previously reported but is clearly visible if the granules are far enough apart. It has the same acidophilic reactions as the granules. The halo which is present only in the early phases of granule formation seems to last but

* The senior author is indebted to Dr. A. B. Sabin, Medical School, University of Cincinnati, for help received and facilities made available in his laboratory during several days spent there while collecting and centrifuging tissues from rabbits inoculated with B-virus and pseudorabies.

a short time, so that in many nuclei the granules completely fill the nucleus.

The granules of herpes and B-virus are slightly basophilic and Feulgen positive, especially in the later phases. Two explanations might be given to account for this basophilia: (1) that the granules are the virus agent and, like the inclusions and elementary bodies of varicella, are Feulgen positive; [25] or (2) that herpes and B-virus are so destructive to the cell that some chromatin liquefies, as it always does in autolysis,[10] and thus imparts to the granules their basophilia. The latter explanation certainly applies to the development of basophilia in inclusions of panleukopenia of cats, and when shrinkage of the nuclear membrane has occurred with loss of the halo the resulting color reactions and morphologic features could not be distinguished in any way from those of a herpetic inclusion. If Figures 33 and 34 had been reproduced in color, they would show the same colors as do Figures 21 and 22. If herpetic granules represent the organism, it might be expected that diploid forms would be discovered. Thus far they have not been observed.

A careful comparison of herpes (Figs. 31, 32, and 33) with B-virus (Fig. 34) has failed to reveal any morphologic or tinctorial differences between the inclusions produced by the two viruses, yet immunologic differences have been established.[26]

A comparison of the inclusions of yellow fever and those found after severe burns was made.* The fully formed inclusion was easily identified in the material from patients who had been burned (Figs. 37, 38, and 39). Early stages may, perhaps, be represented by nuclear reactions of the type shown in Figures 35 and 36, but on the other hand they may represent merely a diffuse hyperoxychromatic reaction which does not lead to typical inclusion formation. It was clearly evident, even with the limited amount of material, that all nuclear reactions do not lead to granular inclusion formation. Sometimes the stimulus produced one to six homogeneous spherical masses in one nucleus, and other "atypical" changes. This variability is reminiscent of some controversies in the literature on yellow fever as to significance of the presence or absence of typical or atypical acidophilic inclusions. The empty nucleus (Fig. 40) is as typical of this material as it is of yellow fever. The structure, apparent developmental cycle, and tinctorial reactions of the inclusions from fatal burns and yellow fever have a

* One of us (A.M.L.) is indebted to Dr. William Boyd of Toronto who kindly sent slides and a block of liver tissue which showed inclusions from cases of burns in man. From some of this material the publication of Dr. Thomas H. Belt[28] was prepared. These were compared with slides of yellow fever in the monkey sent by Dr. E. V. Cowdry of St. Louis.

very close similarity to each other; more so than either of them have to those of panleukopenia of cats or to the inclusions in the Guatemalan amazon,[27] in that neither become basophilic or coalesce into homogeneous masses in their late phases. These observations support the conclusions of Belt [28] that the nuclear inclusions of yellow fever and burns are alike.

Both panleukopenia and the kidney disease of the Guatemalan amazon [27] develop homogeneous masses in the late stages but do it in different ways. The former has already been fully described; in the latter, all of the granules do not coalesce at one time but instead form one or several homogeneous masses embedded in the surrounding granules (Figs. 12 and 13 in the publication by Cowdry, Lucas, and Fox [27]). The material was not adequate to determine whether all of the granules and masses may finally coalesce into one large homogeneous body.

The few comparisons given are enough to indicate that a careful cytologic study of inclusion formation in all of the virus diseases would probably bring out individual differences which might well have some diagnostic value.

Discussion and Conclusions

The group of diseases which morphologically belong to the yellow fever type include, in addition to those already mentioned, Rift Valley fever [29] and perhaps Pacheco's disease of parrots and parakeets. There are probably others if their structure and developmental phases were more exactly known.

The diseases which have been included thus far have one factor in common, and this applies to burns as well: they all tend to dehydrate the animal ~~severely~~. One preliminary experiment was undertaken to determine if dehydration of a healthy cat, by not giving food or water and with frequent doses of ipecac, would produce intranuclear inclusions in the digestive tract. In this single experiment the results were negative, but it should be repeated with a technic which would give more vigorous dehydration of the posterior part of the ileum.

The fact that severe burns will produce intranuclear inclusions nearly identical with those of yellow fever is strong indirect evidence that the virus-produced inclusion is also an oxychromatic segregation. But this does not preclude the possibility that the virus, if present, might be adherent to the oxychromatin granules or embedded within them. The early stages in the formation of inclusion bodies of panleukopenia and other yellow fever types also support the idea of an oxychromatic origin. The later basophilia which may develop in panleukopenia does

not invalidate the idea, because it is fairly certain in this case, as with the submaxillary gland disease of guinea-pigs and ground moles,[30, 31] that it is derived from a solution of basichromatin. Whether basichromatin is the only source of the basophilia in the herpes group of inclusions is still an open question.

Definition of an Inclusion Body

In reviewing the subject of the plasmosome as it reacts in panleukopenia of cats the question is raised, "When is it properly a plasmosome and when a *bona fide* inclusion?" Certainly, structures and nuclear reactions identical with those shown in Figures 14, 15, and 25 have been called inclusion bodies characteristic of virus disease. In panleukopenia of cats, however, they are a minor by-product of plasmosome activity. Also, it has been found in this study that the plasmosome certainly plays no part in the formation of the granular inclusions. It is pushed aside with the basichromatin, and we have concluded, as did Cowdry and Kitchen[12] in yellow fever, that the inclusions which are specific for panleukopenia of cats are not derived from the plasmosome. The question of terminology can probably be answered best in this way: the term, inclusion body, should be applied to those morphologic abnormalities in the cell which, when their complete cycle has been worked out, are found to have a specific or relatively specific association with the agent or pathologic condition which produces them. In panleukopenia, therefore, the plasmosome reactions could not properly be called intranuclear inclusions, but in the guinea-pig after subcutaneous injection of inorganic substances,[15, 32, 33] the same reactions are sufficiently specific for the plasmosome to be called an inclusion body. Likewise, the greatly hypertrophied amphinucleolus which Findlay[34] observed in the livers of a Claxton strain of mice would be considered an intranuclear inclusion although the identical reaction which can occasionally be observed in a normal animal would not be so classified.

This concept of an inclusion body eliminates the difficulties which arise when one defines an intranuclear inclusion as an acidophilic body surrounded by a halo and accompanied by margination of chromatin. It is true that most inclusions which have been described have these characteristics, and it is a useful "rule-of-thumb" to follow, but an analysis of all developmental stages often reveals that these criteria are sometimes applicable only at certain phases of the cycle. Frequently inclusions may be neutrophilic or even fully basophilic at some phase of their development, as are those in fox encephalitis and those described by DeMonbreun and Goodpasture[35] in oral papillomatosis of dogs and as noted by Cowdry, Lucas, and Fox[27] in some kidneys from wild birds and mammals. In some virus diseases the basichromatin does not re-

main marginated against the nuclear membrane but returns to join the surface of the inclusion body. This is true, for example, in late stages in the submaxillary gland disease of guinea-pigs,[13] and occasionally in fox encephalitis.[19] Often the halo is poorly developed or is only transitory as in the diffuse granular inclusions of panleukopenia of cats and late stages of herpes.

It is obvious that in the definition of inclusions which has been suggested there are few morphologic criteria which one can use to determine whether an inclusion body is present in some new virus disease which may be discovered. The burden of proof falls on the investigator to demonstrate that the peculiar cellular reaction is a response which is sufficiently specific for the agent which produces it to have diagnostic value in the identification of that agent. In panleukopenia of cats the diffuse and clustered granular inclusions are specifically diagnostic; all other nuclear reactions which may produce inclusion-like structures are not.*

Summary

1. Examination of cats at intervals during the development of panleukopenia, at the height of the disease and soon afterwards revealed that the primary reactions occur in the lymph nodes and lymphoid tissue of the ileum and are soon followed by reactions in the mucosa of the intestine. The liver, pancreas, and kidney respond late in the disease and in them the resulting injury may be greater than that found in the primary centers.

2. In the lymph glands the initial response, soon after infection, is a draining off of lymphocytes from medullary spaces and cords, accompanied by severe phagocytosis of erythrocytes. The primary nodules do not change extensively during the course of the disease but the secondary centers expand slightly, develop inclusions, and later show some hyaline material.

3. The mucosa of the small intestine is damaged in the terminal portion slightly before the process extends to the duodenum. In both there is extreme destruction of villi and of the epithelium of the crypts of Lieberkühn.

4. Granular intranuclear inclusions have been found in the lymphoid

* The importance of good optical equipment, set up for maximum efficiency, is so important in studies of this sort that it seems desirable to make some mention of it. A Bausch and Lomb ribbon filament lamp, set approximately 25 inches away from the microscope, was used to give Kohler illumination. The condenser of the Zeiss microscope employed was always focused as carefully as the objective. Oil was used between the condenser and slide for critical study. Finally, the apochromatic, 90×, 1.3 n.a. objective employed was of excellent quality. It was compared with other objectives of similar specifications by the same and different manufacturers. Some objectives which were tried failed to separate clearly the individual granules of the inclusion body so that they seemed to run together in a mass, the delicate reticulum could not be clearly distinguished and often fine fibers could not be seen at all.

tissues in 3 days after inoculation but at that time are absent from the intestinal epithelium. In 6 days they are present in both and remain until the height of the disease, after which they all quickly disappear.

5. Examination of clinic animals would indicate that most of them died after the crisis had passed and that inclusions and initial reactions of lymphoid tissue, characteristic of the disease, were absent; instead, there was pronounced damage to the secondarily reacting tissues.

6. Two types of granular intranuclear inclusions were present, both in lymphoid tissue and intestinal epithelium: one has been called *clustered granular,* and the other *diffuse granular.* The developmental cycle of each has been worked out. With ageing they develop a slight basophilia superimposed on an original, strong eosinophilia and in the late stages become homogeneous in character.

7. In liver as well as in lymphadenoid and intestinal tissues homogeneous inclusions derived from plasmosomes may be found. Homogeneous inclusions derived from the granular types may be distinguished from those derived from plasmosomes by a difference in tinctorial reaction in most cases.

8. Two cells with inclusions were found which were undergoing mitosis; one was in the prophase and the other in what might be a metaphase. This is the first report of mitosis in cells bearing intranuclear inclusions of the granular type.

9. On the basis of morphology and tinctorial reactions, granular inclusions of panleukopenia of cats have been classified with those of yellow fever and burns, but differences in their developmental cycles have been pointed out. The work of Belt,[28] showing that the inclusions of burns are similar morphologically to those of yellow fever, has been confirmed.

10. By way of definition, the term, inclusion body, should be applied to those morphologic abnormalities in the cell which, when their complete cycle has been worked out, are found to have a specific or relatively specific association with the agent or pathologic condition which produces them.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

Drawings were outlined with a camera lucida at a projected magnification of × 3250 and have been reduced one-fourth in publication.

PLATE 78

All cells are from tissues fixed in Zenker's solution with acetic acid, except where noted, and stained with hematoxylin and eosin. *Figures 1 to 30 inclusive* are of cells from normal cats or those having panleukopenia.

FIG. 1. Cat 81. Epithelium, lower ileum. Hyperoxychromatism, which falls within the range of usual nuclear variation.

FIG. 2. Cat 80. Lymph nodule, ileocecal junction. Early stage in clustered granular inclusion.

FIG. 3. Cat 82. Epithelium, lower ileum. Early stage in inclusion formation.

FIG. 4. Cat 82. Epithelium, lower ileum. Coloration of inclusions characteristic of young to fully formed inclusions.

FIG. 5. Cat 81. Epithelium, lower ileum. Fully formed inclusion. Slight basophilia superimposed on original acidophilia.

FIGS. 6 and 7. Cat. 81. Epithelium, lower ileum. Late homogeneous stage of inclusion cycle. Figure 7 has a neutrophilic plasmosome to the left of the inclusion.

FIG. 8. Cat 80. From lymph node near colon. Probably early stage in formation of diffuse granular inclusion.

FIG. 9. Cat 81. Epithelium from duodenum. Young but clearly recognizable diffuse granular inclusion.

FIG. 10. Cat. 80. From lymph node near colon. Fully formed diffuse granular inclusion.

FIG. 11. Cat 80. From lymph nodule, lower ileum. Slight basophilia and condensation, evidences of ageing.

FIG. 12. Cat 81. Epithelium, duodenum. Late stage in diffuse granular inclusion. Corresponds to Figure 6 for the clustered granular inclusion.

FIG. 13. Cat. 81. Epithelium, duodenum. Nucleus shows characteristics of both diffuse and clustered inclusions.

FIG. 14. Cat 82. Epithelium, lower ileum. A neutrophilic plasmosome surrounded by a halo, chromatin partially marginated.

FIG. 15. Cat 82. Epithelium, lower ileum. A younger acidophilic and an older neutrophilic plasmosome.

FIG. 16. Cat 82. Lymph nodule, lower ileum. Neutrophilic bodies embedded in oxychromatin. Probably an atypical plasmosome.

FIG. 17. Cat 82. Lymph nodule, lower ileum. Homogeneous body intermediate in color between homogeneous stage of granular inclusion and neutrophilic plasmosome. Origin, therefore, not known.

FIG. 18. Cat 82. Epithelium, lower ileum. Prophase stage of mitosis in a nucleus which contains a clustered granular inclusion.

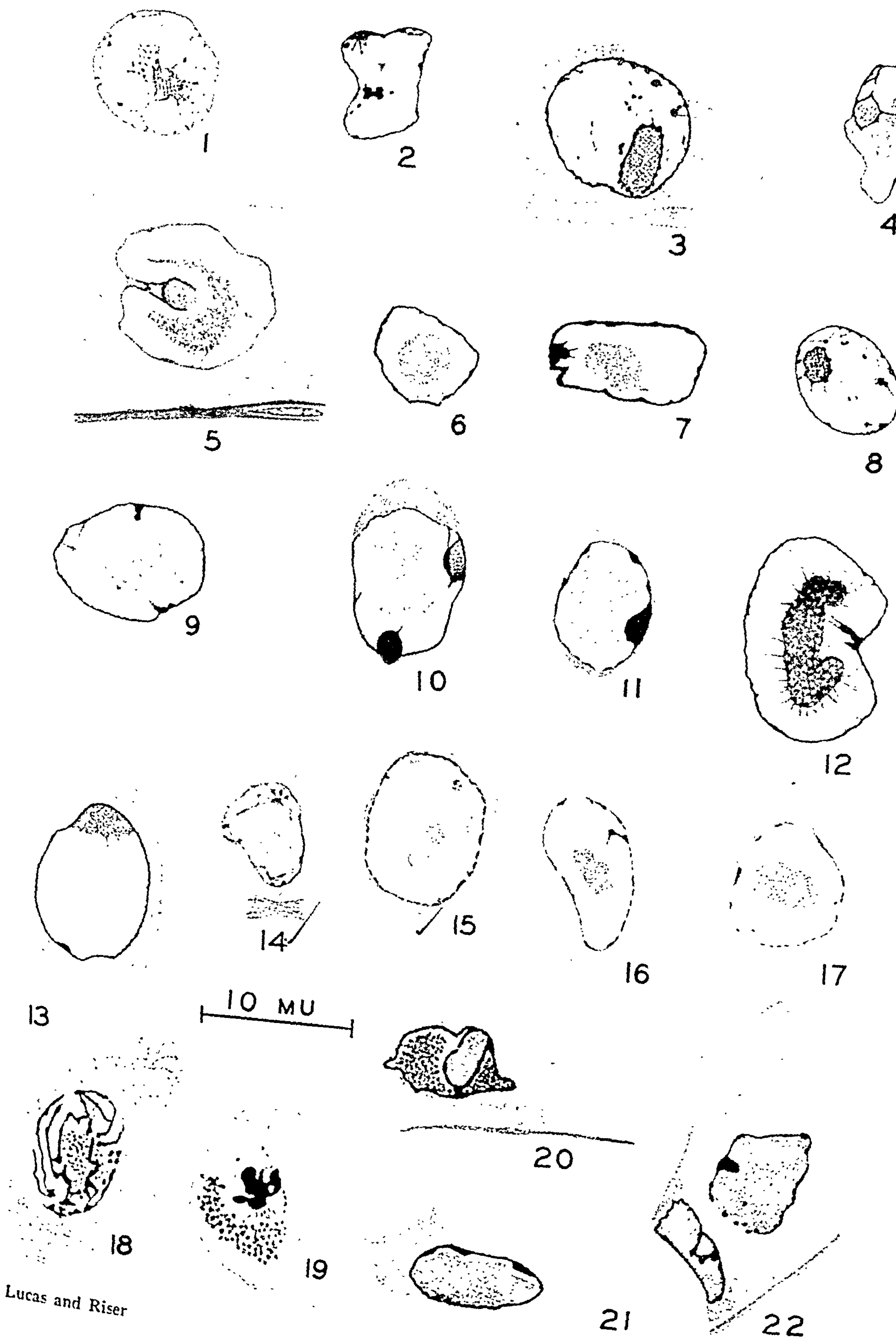
FIG. 19. Cat 81. Epithelium, duodenum. Late prophase or metaphase of a nucleus with an intranuclear inclusion. Mitosis probably abnormal.

FIG. 20. Cat 82. Epithelium, lower ileum. Beginning necrobiotic changes in a nucleus which has a clustered granular inclusion in middle phase of its cycle.

FIG. 21. Cat 82. Epithelium, lower ileum. Late necrobiotic changes in nucleus with older, but not yet homogeneous, granular inclusion. Ballooned, striated, cuticular border.

FIG. 22. Cat 82. Epithelium, lower ileum. Nucleus above shows karyorrhexis of chromatin. Halo gone. In color and structure resembles late herpes inclusion. Nucleus below shows chromatin in solution. Inclusion may or may not have been present in the latter.

PLATE 78



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Intranuclear Inclusions in Panleukopenia

FIG. 23. Cat 82. Liver cell. Typical nuclear structure. Located three cells away from one shown in Figure 25.

FIG. 24. Cat 82. Liver cell. Partial retraction of chromatin from plasmosome; cell is not preparing for mitosis.

FIG. 25. Cat 82. Liver cell. Plasmosome surrounded by halo and partially marginated chromatin. Structurally identical with intranuclear inclusions associated with some virus diseases.

FIG. 26. Cat 81. Lymph node near colon. Hypertrophy of a plasmosome with vacuolization accentuated by refractility of the margins or substance within the vacuole.

Figures 27, 28, and 29-a were drawn to show all structures of the dorsal half of the nuclear sphere.

FIG. 27. Cat 79. From lymph nodule in ileocecal junction. Early prophase in mitosis. With the formation of the spireme, the basichromatin leaves the plasmosome.

FIG. 28. Cat 79. From lymph nodule in ileocecal junction. Prophase, later than cell in Figure 27. Most of the spireme threads have left the plasmosome and gone to the nuclear margin.

FIG. 29-a. Cat 79. From lymph nodule in ileocecal junction. Prophase, later than Figure 28. All chromatin has left the plasmosome except one strand.

FIG. 29-b. Same nucleus as shown in Figure 29-a, but drawn at a fixed level of focus. Simulates an intranuclear inclusion, with halo and marginated chromatin.

FIG. 30. Cat 33. Epithelium, ileum. Empty or ghost nucleus. Margination of chromatin but no inclusion. All but a small portion of this particular nucleus was contained in a single section.

Figures 31, 32 and 33 are drawings of cells from rabbit brain showing inclusions of herpes simplex.

FIG. 31. Early nuclear reaction, margination, and oxychromatic accumulations.

FIG. 32. Oxychromatic masses and accumulating granules characteristic of the typical inclusion. Reticulum joining granules is occasionally visible.

FIG. 33. Late inclusion. Homogeneous masses and halo gone. Granules joined by reticulum fill the nucleus.

FIG. 34. Testis of rabbit inoculated with B-virus. Small blood vessel surrounded by inclusion-bearing cells. Early stage, cells at lower right and left corners; homogeneous oxychromatic masses with granules. Middle stage, upper cell; medium halo, granules on conspicuous reticulum. Later stage, middle right; halo almost gone; granules more numerous and closer but reticulum still clear.

Figures 35 to 40 are drawings of liver cells from human cases of severe burns. Formalin fixation. Case numbers are from the pathology records of the University of Toronto. A-39-32: F., 51 years. Burn from wax, died 3 days later. A-157-34: M., 37 years. Burn from gasoline, died 3 days later. A-306-34: M., 55 years. Burn from steam, died 2 days later.

FIG. 35. A-306-34. Most of the chromatin has marginated. Some lagging of the amphinucleolus. Scattered clusters of acidophilic granules.

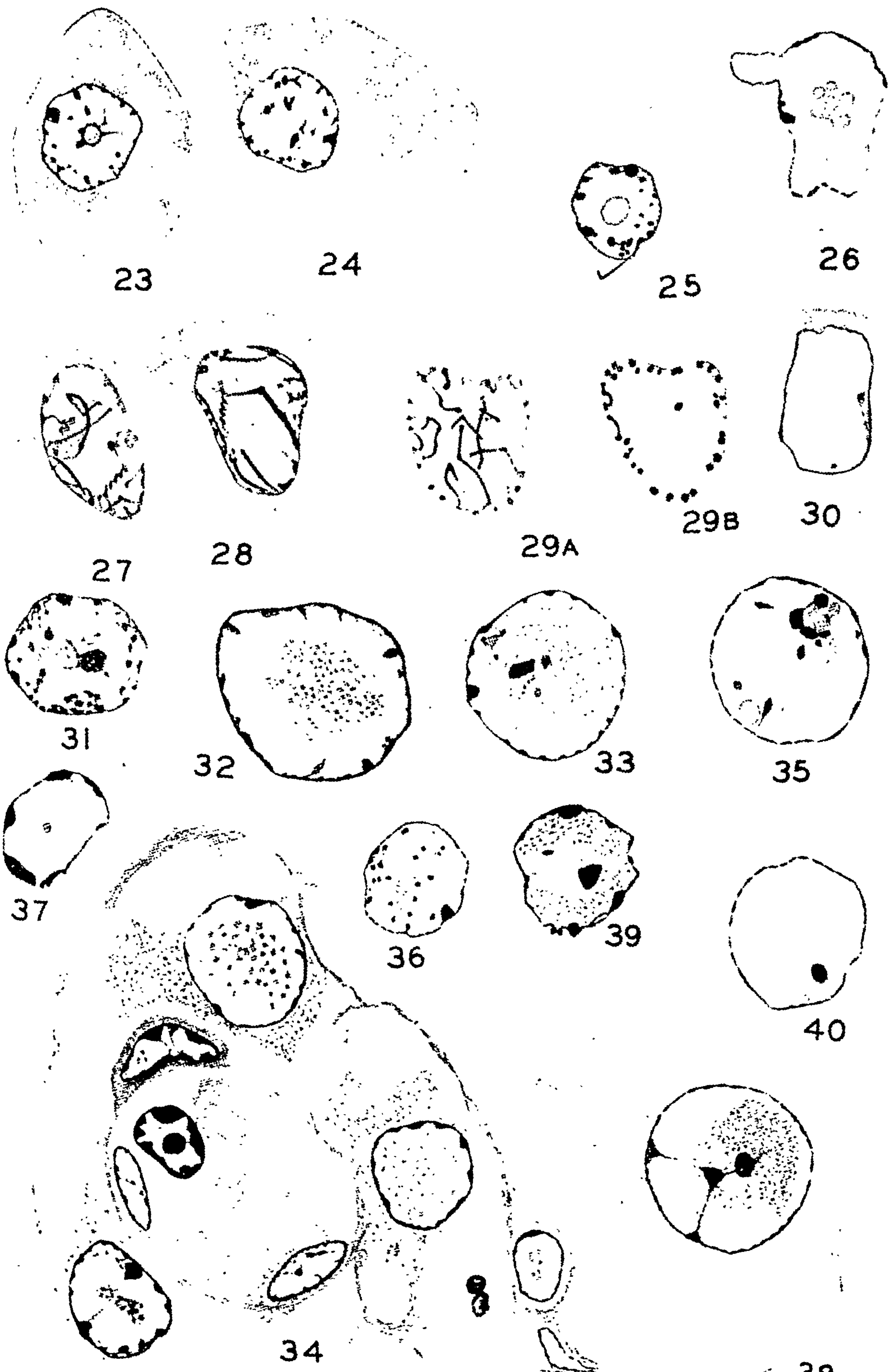
FIG. 36. A-157-34. A few basichromatin granules associated with acidophilic clusters.

FIG. 37. A-39-32. Early karyorrhexis of marginated basichromatin. Oxychromatin clustered in center to form inclusion body. Similar to yellow fever inclusion.

FIG. 38. A-306-34. Resembles closely a fully formed yellow fever inclusion. Granules slightly less refractile in material available for comparison.

FIG. 39. A-39-32. Nuclear death superimposed on inclusion. In addition to shrinkage and beginning karyorrhexis, there is liquefaction of basichromatin, which causes inclusion to resemble that of herpes.

FIG. 40. A-306-34. Empty or ghost nucleus, often found in this material and in yellow fever.



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Intranuclear Inclusions in Panleukopenia

SARCOSPORIDIOSIS OR TOXOPLASMOSIS IN MAN AND GUINEA-PIG *

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INTRODUCTION

In many of the texts on human parasitology is a chapter entitled "Parasites of Undetermined Nature," and according to Craig and Faust [1] "Those belonging to the Sarcosporidia and to Toxoplasma are the most important . . ."

The exact taxonomic status of the Sarcosporidia has not been determined. The order Sarcosporidia, Bütschlii, 1882, containing only one genus, Sarcocystis, Lankester, 1882, has been considered closely allied to the Cnidosporidia [2] or Neosporidia,[3] but Wenyon [4] disapproved of this classification. Belding [5] included the order Sarcosporidia in the subclass Acnidosporidia (characterized by the production of simple spores during the life of the sporozoon), class Sporozoa. Approximately 50 species of Sarcocystis have been recorded in mammals, including man, rat, mouse, sheep, cattle, pig, horse, rabbit, dog, cat, deer; birds; reptiles; and recently in fish.[6] It is probable that there are fewer species, although Alexeieff's [7] suggestion that only one species exists may be too radical.

The epidemiology and life cycle of Sarcosporidia are incompletely known. The evidence suggests that, following the consumption of infected food, sporozoites are liberated from the adult cysts, penetrate the intestinal epithelium, reach the lymphatics or possibly the blood stream, and eventually localize in the skeletal muscles. Animals on the whole are tolerant to the presence of sarcocysts, although various debilities have been associated with the infection (see Scott [8] for review). Pfeiffer [9] first demonstrated that emulsions of the parasite of sheep, *Sarcocystis tenella,* when injected subcutaneously, can kill mice and guinea-pigs. The name of "sarcocystin" was applied to this toxic substance by Laveran and Mesnil.[10]

In his authoritative monograph on the Sarcosporidia, Babudieri [11] accepted the following as authentic reports of human sarcosporidiosis:

Baraban and St. Remy [12]	1894	Manifold [16]	1924
Vuillemin [13]	1902	Lambert [17]	1927
Darling [14]	1909	Vasudevan [18]	1927
Darling [15]	1920	Bonne and Soewandi [19]	1929
To these may be added:			
Feng [20]	1932	Hewitt [22]	1933
Price [21]	1933	Gilmore, Kean and Posey [23]	1942

* Received for publication, June 5, 1944.

We are in agreement with Babudieri [11] in regard to these cases which he regarded as authentic (within the limitations of the discussion in this paper) and also with his rejection of many reported cases because he considered them incorrect, doubtful, or lacking in sufficient data for review. The latter group includes the cases of Lindemann, Leuckart, Koch and Gaffky, Rosenberg, Kartulis, and others. (See Babudieri for review.) The case of Kartulis [24] has in the past been rejected by some authorities and credited by others. We are inclined to believe that this is an authentic case, but proof at this date seems impossible.

The case of "bone sarcosporidiosis" reported by Cone [25] must be questioned. In 1928, Naidu [26] published a record of a patient who probably was the same one described by Vasudevan [18] in 1927. The infection observed by Hertig [27] in the myocardium of an infant has been redescribed by Pinkerton and Weinman [28] as toxoplasmosis. Price [21] noted cases of "Scoglia" (probably Scaglia [29]) and of Askanazy,[30] and Rivas [31] mentioned those of Nancy and A. J. Smith, but we have been unable to find records of these cases.

Morphology of the Parasites Hitherto Reported as Sarcosporidia in Man

Although the morphology of the Sarcosporidia varies considerably depending upon the species(?), host, and age, the following description by Craig and Faust [1] of the human parasite, *Sarcocystis lindemanni*, Rivolta, 1878, is generally applicable:

"When fully developed, *S. lindemanni* consists of a cylindrical, elongated or fusiform body, hyaline in appearance, with more or less pointed ends, lying in the affected muscle fibers. It is enclosed in a membrane and contains myriads of round and crescent-shaped spores. In 1843, Miescher found these bodies in the muscle fibers of mice; since that time they have been known as 'Miescher's tubes.' They vary much in size, from forms measuring as much as 5 centimeters in length to others so small that it requires a microscope to detect them. When visible to the naked eye they appear as minute white streaks within the muscle fibers. When removed from a muscle fiber each parasite consists of a cylindrical whitish tube with somewhat pointed ends and a finely lobulated surface. The membrane forming the outer coat of the organism may show a radial striation and from this membrane firm prolongations extend, dividing the tube into separate compartments, of which the outer contain round cells, while in the inner, fully developed compartments there occur the characteristic crescentic bodies called spores. Rounded, ovoidal, elongated or sickle-shaped spores are produced which are known as 'Rainey's corpuscles.' The spores measure from 12 to 16 microns in length and from 4 to 9 microns in breadth; when properly stained they are seen to contain an elongated nucleus situated near the more rounded end of the spore. This nucleus consists of a nuclear membrane and a central karyosome. Many thousands of spores are contained in a fully developed parasite."

In 1894 Baraban and St. Remy [12] described the first case of human sarcosporidiosis which has been universally accepted as authentic. The

parasites were found in the laryngeal muscles of a man executed by hanging. The parasites were large, measuring 1.6 mm. by 0.17 mm., and distended the muscle fibers. The capsules were thin, but striated. Room compartments contained individual sporozoites measuring 8 to 9 μ in length. No inflammatory reaction was present.

Darling,[14] in 1909, recorded what he considered the third instance of human sarcosporidiosis. He was unaware, apparently, of Vuillemin's [13] report but accepted at that time the case of Kartulis.[24] The parasites were found in the biceps of a Negro ill of typhoid fever. Darling's observations may be recorded in detail:

"Here and there were oval or round dotted bodies about the width of a striated muscle fiber, their length being about twice that. The muscle fiber was not distended by the presence of the dotted body. One oval body measured 0.084 mm. in length and 0.027 mm. in width. One of the bodies in cross section was circular and was 0.021 mm. in diameter. The oval or round bodies were strongly contrasted from the eosin-staining muscle fiber by the very pronounced blue stippling of the former. This stippling was seen under the high power to be due to the nuclei of small oval sporozoites. In this section there was some hyaline change in the muscle fibers, both in the infected and the non-infected fibers. Occasionally within the capillaries near one of the oval bodies there was a slight increase in the number of polymorphonuclear leucocytes and there were a few foci of acute myositis involving a single fiber. Some of the specimens showed a cross-section of the sporozoön, in which it was seen that the latter was just within the sarcolemma. No matter how wrinkled or distorted the muscle fiber might be, the sporozoön, with its very thin refractile membrane, was seen to preserve its circular or oval outline. Under the highest powers the sporozoön was seen to be made up of hundreds of little oval vesicular bodies having a round nucleus at one end. The sporozoite took the eosin irregularly and appeared to be vacuolated. The little sporozoites were decidedly vesicular and were either round or oval. All had one nucleus and very rarely two nuclei placed at opposite ends of the short axis of the sporozoite. The sporozoites were closely packed within the mother capsule or membrane, yet without any arrangement suggesting a room system. The measurement of sporozoites in the section was: length 4.25 microns, width 1.75 microns."

It was obvious to Darling [14] that the parasite he observed differed in several respects from that described by Baraban and St. Remy [12] and also from animal Sarcosporidia such as *Sarcocystis tenella* (in sheep) (Fig. 4) and *Sarcocystis miescheriana* (in pigs) (Fig. 5). Darling believed that the parasites in his human case differed from those in animals because man was a strange host and hence the parasite was unable to complete its life cycle.

The experiments of Darling [32] presented what has been considered proof of his theory. He fed 6 guinea-pigs rat muscle naturally infected with *Sarcocystis muris,* and found that 2 pigs killed 6 months later had parasites in the skeletal muscles similar to those seen in his human case. These uncontrolled experiments were supposed to have confirmed the work of Negri,[33] who fed 11 guinea-pigs infected rat muscle and found parasites in 9. No parasites were found in 12 control pigs. Negri was

impressed by the differences between the parasites in his experimental animals and in rats, and attributed the morphologic change to the fact that the parasites were in a strange host, a theory popularized by Darling. Both Negri and Darling used the guinea-pig as their experimental animal because they believed that this animal was not naturally infected with Sarcosporidia.

Our review of the reported cases of human sarcosporidiosis has revealed that they may be divided into two groups: Group I in which the parasites, cyst and individuals, are large, with well defined, striated cyst walls and inner room compartments. This parasite resembles the Sarcosporidia of animals such as sheep and pig. In this category belong the cases of Baraban and St. Remy,[12] Vuillemin,[13] Bonne and Soewandi,[19] Feng,[20] Price,[21] and Hewitt.[22]

Group II is characterized by the smaller size of the cyst and its individual members, a delicate, ill defined, nonstriated capsule and the absence of internal septa. Here may be placed the two cases of Darling[14, 15] and those observed by Manifold,[16] Lambert,[17] Gilmore, Kean, and Posey.[23]

Until a few years ago the following case would surely have been listed as another instance of sarcosporidiosis and, according to the foregoing classification, would belong to group II.

Report of Case

Patient A. W. (Gorgas Hospital chart no. 530409, Board of Health Laboratory autopsy no. 14230) was a Negro housewife, 48 years old, who died in Gorgas Hospital, Ancon, Canal Zone, on August 17, 1943, 4 hours after admission for acute dyspnea and retrosternal pain.

Background. The patient was born in Jamaica, British West Indies, and lived there until the age of 25. The family history was not contributory. At the age of 25 she went to Bocas del Toro, Republic of Panama, where she worked as a seamstress for 12 years, then spent some years in Almirante and Puerto Armuelles, Republic of Panama, and came to the city of Panama about a year before her death. Her health had generally been good. She had had a child which died in infancy before she left Jamaica. She married in Bocas del Toro at about the age of 28 years and had two abortions in the early months of pregnancy.

A year before her death she was admitted to The United Fruit Company Hospital in Puerto Armuelles, Republic of Panama, where a colpotomy for pelvic abscess was performed. Convalescence was uncomplicated. No history of antiluetic therapy was obtained. Her food habits were not remarkable and were typical of Jamaicans. She ate no raw meat. Her only obvious contact with animals was with a dog, a household pet not available for examination. It was known that the house in which she resided in Panama during the last year of her life was infested with rats, and contamination of food was possible. Her husband was living and well, and declined to permit a muscle biopsy.

History of Terminal Illness. Three weeks before admission the patient became ill, complaining of epigastric pain, exertional dyspnea, orthopnea, and swelling of the right leg. She continued her household duties for a fortnight, however, but then took to bed. On the day of admission, sharp, severe, substernal pain radiating to

the back, marked dyspnea, abdominal distention, profuse diaphoresis, and questionable fever brought her to the hospital.

Physical Examination. The patient was a somewhat obese Negro woman in acute distress. Temperature was 97.0° F.; pulse rate, 120 per minute; respiration, 32 per minute; blood pressure, 120 mm. Hg systolic and 90 mm. Hg diastolic. A systolic murmur was heard at the apex of the heart, but no other abnormalities were noted upon physical examination of the heart and lungs. No peripheral edema was present.

Course in Hospital. One hour after admission the retrosternal pain became even more severe and she vomited. She was given morphine sulphate, grains 1/6, without relief. Two hours after admission she appeared to be in extreme shock. Her skin was cold and clammy, the blood pressure could not be obtained, the heart sounds were poor in quality and the cardiac rhythm became totally irregular. She died 4 hours after admission without rallying. No laboratory procedures were conducted. The clinical diagnosis was coronary occlusion.

AUTOPSY FINDINGS

A complete autopsy, including examination of the head, was performed 11 hours after death. The anatomic diagnoses included: parasitization of heart (sarcosporidiosis or toxoplasmosis); cardiac hypertrophy (360 gm.); chronic interstitial myocarditis, slight; area of focal encephalomalacia of brain, small; chronic diffuse thyroiditis (Hashimoto type); hemorrhages of thymus, small, focal, terminal; acute and chronic passive congestion of liver; fibrous perisplenitis, old; fibromyomata of uterus; chronic pelvic peritonitis; chronic salpingitis, bilateral; atrophy of ovaries; obesity, moderate; general arteriosclerosis, slight; post-mortem degeneration of pancreas and kidneys, moderate. The exact cause of death was not determined nor could the pathologic findings be correlated satisfactorily with the clinical course.

Since the parasites were observed only within the heart, description will be limited to that organ. No skeletal muscle was taken for examination. No parasites were found in 167 sections of the brain.

Gross Examination of the Heart

No excess pericardial fluid was present. The heart was slightly enlarged, weighing 360 gm. after removal of blood clot from the chambers. The apex was located in the fifth interspace, 11.0 cm. to the left of the midsternal line. The epicardial surface was smooth and free from adhesions to the pericardium. A moderate amount of subepicardial fat was present. The myocardium was uniformly firm and reddish brown. No areas of myocardial necrosis or fibrosis were recognized. The endocardium was smooth and glistening. The valve leaflets were delicate and appeared competent. No lesions of the aortic valves suggestive of syphilis were present. Coronary sclerosis was slight; no occlusion was observed.

Microscopic Examination of the Heart

Thirteen blocks were taken from the interventricular septum, the left ventricular wall, and the right ventricular wall. Sections of the auricular walls were not taken. From these thirteen blocks, 365 sections were prepared. In six of the blocks, including all taken from the right ventricular wall, no significant abnormalities were noted. The subepicardial fat was free of inflammatory reaction. The myocardial fibers tended to be large and contained a small to moderate amount of yellowish perinuclear pigment. A few small patches of myocardial fibrosis were found. The endocardium was not thickened and no verrucae were seen.

In seven sections of the left ventricular wall there were found nine parasitic cysts. Since all of these cysts were similar in character, it may be sufficient to describe a typical one (Fig. 1).

Within a single swollen muscle fiber, cut in cross or slightly tangential section, was a cyst measuring 60 by 50 μ. The cyst was packed with numerous elongated, slightly elliptical bodies which, for purposes of convenience in description, were called sporozoites. These bodies averaged 5 μ in their greatest dimension and had nuclei approximately 1 μ in diameter generally located at one pole. The cyst possessed a delicate capsule, or membrane, which stained poorly in routine hematoxylin and eosin sections, but in van Gieson preparations stood out as a shiny, refractile structure. It could not be ascertained whether the capsule was of parasitic or muscle-fiber origin. No striation of the capsule and no internal septa could be demonstrated. Toward the periphery of the cyst, just beneath the capsule, elongated sporozoites were not seen. Instead, there was a row of tiny nuclei representing, possibly, immature sporozoites.

The following were measurements of other cysts: 50 by 27 μ; 105 by 47 μ; 80 by 52 μ; 35 by 26 μ. Attempts to section, in serial fashion, some of the parasites were productive of the following results. In most instances serial sections could not be obtained, for the parasites were found in only one slide. In one instance, 15 consecutive sections, cut at intervals of 5 μ, included the same cyst. It was estimated, therefore, that the size of this cyst was at least 75 by 35 by 25 μ. In another instance it was possible to obtain 9 consecutive sections, each containing the same cyst, and from the method of preparation of this material and from a study of the sections it appeared likely that the entire parasitic structure was included in the material studied. The dimensions of this cyst were 105 by 47 μ.

A striking feature of the sections was the virtual absence of inflam-

matory reaction in the myocardium adjacent to the parasites. In fact, in only one section of the left ventricular wall could an area be found in which there was a distinct chronic inflammatory reaction with infiltration by lymphocytes. Scattered within other sections, however, there could be found a slight inflammatory reaction which bore no direct relation to the presence of the cysts. No spirochetes were found in sections stained by Levaditi's method.

Several of the cysts were ruptured by microdissection of stained material and the individual parasites studied. These averaged 5 by 1 μ and were characterized by an elongated, elliptical or crescentic shape, and by proportionately large nuclei which, for the most part, were located at one pole and produced a bulge in the outline of the sporozoite. The illustrations published by Darling [32] of the sporozoites seen in his "experimental guinea-pig sarcosporidiosis" are accurate representations of the individual sporozoites in this human material.

Parasites in Guinea-Pig

In 1942 Gilmore, Kean, and Posey [23] reported the presence of parasites in the heart of a Panamanian girl, 12 years old, which morphologically appear identical with those in the current case. At that time, following suggestions by Augustine [34] and Weinman,[35] serious consideration was given to the possibility that the parasite was Toxoplasma. The parasite was classified as Sarcosporidia, however, for various reasons which are recorded in the report; among those reasons was the fact that the parasite was indistinguishable from those in Darling's [15, 32] illustrations and those of subsequent cases,[16, 17] the diagnosis of which had not been questioned.

When the current case appeared we determined to investigate the muscles of guinea-pigs, for if Darling were wrong in his classification, then all cases in group II (page 470) were in an incorrect category. It did not seem reasonable that Sarcosporidia, which is so widespread in its distribution, should be absent in the guinea-pig.

Between September 10, 1943, and November 13, 1943, the skeletal muscles of 60 laboratory guinea-pigs, *Cavia cobaya,* were examined. These pigs were imported from the United States where they had been purchased from a commercial dealer; presumably they had not been used previously for experimental purposes. They were kept under the usual laboratory conditions until sacrificed for complement. No muscle parasites were recognized grossly. Blocks of thigh and abdominal muscles of all were fixed in formol-alcohol, and prepared by the usual paraffin method, with hematoxylin and eosin staining.

In 5 of the 60 animals parasites resembling those described and illustrated by Negri [33] and Darling [14] were found in the skeletal muscles. Complete autopsies were done on 2 of these 5, the bodies of which had been saved in the ice box. In 1 of these 2 guinea-pigs parasites were found only in the thigh muscles. Examination of the masseter, pectoral, abdominal, and tongue muscles, and of brain, heart, lungs, liver, spleen, and kidneys was negative. In the other pig parasites were found in thigh and paravertebral muscles, brain, and kidney; no parasites were noted in sections of heart, lungs, liver, spleen, tongue, esophagus, and trachea.

MUSCLE

The parasites were not significantly different in any of the 5 pigs in which they were found. About half of the muscle sections of these pigs had cysts. The parasites were more numerous in sections of the thigh than in the other muscle groups such as abdominal, pectoral, and masseter. The greatest number found in any one section was three well formed cysts (Fig. 2). The cysts were located within individual muscle fibers, generally in an eccentric position. In a few instances the cysts seemed to be located between muscle fibers.

Cross and tangential sections of cysts varied in size from 20 by 26 μ to 48 by 18 μ. Since none of the cysts was cut longitudinally, their length was not determined. In one instance it was possible to obtain six serial sections cut at intervals of 5 μ, which would make the cyst at least 30 μ long. Most of the cysts were cut in cross section and appeared as circular nests; others were cut obliquely and were ovoid in shape. The parasitized muscle fibers were swollen and a few showed hyaline degeneration. Each cyst possessed a thin, delicate capsule or limiting membrane measuring approximately 1 μ in thickness. This membrane could be seen best in sections stained with van Gieson's picro-acid fuchsin by which it was stained red and stood out in marked contrast to the greenish yellow fiber. Sarcolemma, however, possessed the same tinctorial properties. We could not determine whether the capsule was of parasitic or muscle origin.

Each cyst was composed of, or filled with, many tiny bodies which, for purposes of convenience, were termed sporozoites. Under the low power objective the sporozoites were noted as fine basophilic stippling within the cyst. Under higher magnification the sporozoites were seen to be elongated. They were so closely packed that counting was difficult but it was estimated that each cyst contained about 150 sporozoites. In some of the cysts the bodies were disposed in a haphazard fashion, whereas in others they tended to collect in groups. Toward the periphery of the cysts, the elongated bodies were not present. Instead,

tiny spherical or ovoid basophilic bodies resembling nuclei of the sporozoites were noted.

In order to study the individual sporozoites, the following technic was employed. The coverslip was removed from a stained section and a drop of balsam was placed on the tissue. Practically all of the tissue surrounding a cyst was dissected away and the débris was flushed off with xylol. A tiny drop of balsam was placed directly upon the cyst and the coverslip returned. Pressure on the coverslip was applied. This was sufficient to rupture the cyst capsule and the extruded individual sporozoites could then be examined under the oil immersion objective and measured. The dangers of regarding the measurements of sporozoites treated in such a fashion as *in vivo* dimensions must be obvious.

In these preparations the sporozoites measured 5 by 1 μ. Some were crescentic or banana-shaped. Most of them tapered at both ends, but several were bluntly rounded at one pole. A dark-staining nucleus was present near the tip of each parasite, and in many it was found to occupy the extreme distal part of the sporozoite. In these sporozoites the nucleus was found to accommodate itself to the taper of the corpuscle and appeared as a dark-staining, roughly triangular body with a somewhat rounded base. The nuclei in other sporozoites were found to be nearer the center, but it was difficult to find any with a centrally placed nucleus. A section stained by Heidenhain's iron hematoxylin technic showed the nuclear chromatin to be arranged chiefly around the periphery of the nucleus. No definite intranuclear structures were ascertainable. The cytoplasm of some of the corpuscles was distinctly granular, and vacuoles were present in a few of them. The morphologic characteristics of the cysts and of sporozoites appeared identical with those so beautifully illustrated by Darling.[14, 32]

BRAIN

Parasites were found in the brain of 1 of the 2 guinea-pigs upon which complete autopsies were done. The cysts (Fig. 3) were ovoid or circular in outline and measured from 20 to 25 μ in diameter. No outer membrane or capsule could be recognized and the cysts seemed to be limited only by the surrounding parenchyma. As many as 80 sporozoites could be counted in some collections; these resembled those found in the muscles, but appeared scattered in an ill defined, faintly basophilic ground substance. There was no surrounding cellular reaction, but the leptomeninges showed a slight chronic inflammatory exudate. These cysts resembled the illustrations of toxoplasmosis in guinea-pigs (Markham [36]), wild rats (Perrin, Brigham, and Pickens [37]), and mice (Weinman [38]).

KIDNEY

Within the lumen of a renal tubule was a circular nest of parasites measuring 35 μ in diameter. This nest was composed of 50 or 60 nucleated bodies scattered in faintly basophilic stroma. Nearby was a collection of 7 smaller cysts ranging from 15 to 20 μ in diameter. Each of these contained 10 to 15 nuclear structures scattered in a similar stroma. These aggregates resembled those found in the brain and muscles, but there was less differentiation of the internal structure. Post-mortem degeneration was considerable and may have played a rôle in obscuring the inner structure of these cysts. There was no surrounding inflammatory reaction.

COMMENT

In the absence of inoculation and serologic studies, absolute identification of the parasites in the current human case and in the guinea-pigs is impossible. On morphologic grounds, however, the following statements appear warranted:

1. The parasites in our human case are similar to those described by Darling and others (group II) as Sarcosporidia.

2. The classification of these parasites as Sarcosporidia has been based mainly upon Darling's experiment. We found parasites occurring spontaneously in guinea-pigs similar to those which Darling thought he had transmitted experimentally. Negri's work [33] requires confirmation.

3. The parasites in both group II and in the guinea-pigs resemble Toxoplasma more than Sarcosporidia. Toxoplasmosis has been reported in guinea-pigs by Mooser,[39] Markham,[36] and Sabin and Olitsky,[40] but parasites in peripheral skeletal muscle were not mentioned. A detailed discussion of Toxoplasma in man need not be presented for several articles have summarized current knowledge (Pinkerton and Weinman,[28] Wolf, Cowen, and Paige,[41] etc.). The cysts, both in group II and in guinea-pigs, are somewhat larger than those generally described in chronic toxoplasmosis,[37,38] possibly because they are present in the more abundant cytoplasm of striated muscle.

4. Although the parasites in group II of the human cases and in the guinea-pig appear similar, it has not been established that they are identical. In fact, there is no proof that the parasites in the muscles, brain, and kidney of the guinea-pig are the same.

It is of some interest that three instances of this type of human infection should have been found in one laboratory when so few cases have been recorded all over the world. Darling [14] reported in 1909 the parasites in the biceps muscle of a patient ill of typhoid fever. The

second case (Gilmore, Kean, and Posey[23]) was found in 1941 while searching for *Trypanosoma cruzi* in the heart of a child. In the current case many blocks of heart muscle were taken because Chagas' disease was suspected at the autopsy table but the parasites were first seen by a technician (Mr. J. M. Benevides) who picked up a slide to check its stain. (Darling's second case[15] was from the Federated Malay States.) *

Summary

1. The literature on human sarcosporidiosis was reviewed and the reported cases were divided into two groups:

Group I, in which the parasites were characterized by the large size of the cysts and sporozoites, striated capsules, and internal septa. This group resembled animal Sarcosporidia such as *Sarcocystis tenella* (sheep) and *Sarcocystis miescheriana* (pig). These were regarded as authentic cases of human sarcosporidiosis.

Group II, in which the parasites were characterized by smaller size of the cyst and sporozoites, and absence of striated capsule and internal septa. These cysts resembled parasites which Darling thought he had transmitted to guinea-pigs by feeding them rat muscle infected with *Sarcocystis muris*.

2. A case is reported in which parasites in the heart of a Negro woman belonged in group II.

3. The spontaneous occurrence in the skeletal muscle of 5 of 60 guinea-pigs of parasites morphologically indistinguishable from those which Darling claimed to have transmitted experimentally was observed. The presence of parasites resembling Toxoplasma in the brain and kidney of one of these pigs suggested strongly that the muscle parasites were also Toxoplasma.

4. Since the classification of group II as Sarcosporidia stemmed from Darling's questionable experiment, it was considered probable that the parasites in group II were Toxoplasma rather than Sarcosporidia.

5. In the absence of serologic and inoculation experiments, final classification of the parasites was not attempted. The possibility that the parasites in group II and in the muscle of guinea-pigs were neither Sarcosporidia nor Toxoplasma was not excluded.

We are indebted to Dr. Carl M. Johnson, Gorgas Memorial Laboratory, Panama, R. of P., for the photomicrographs.

* Since this paper was submitted for publication, parasites resembling Toxoplasma were found in sections of the brain and heart of a child upon whom an autopsy was performed in this laboratory in 1936. (Tomlinson, W. J. Human chronic toxoplasmosis. *Am. J. Clin. Path.* In press.)

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[*Illustrations follow*]

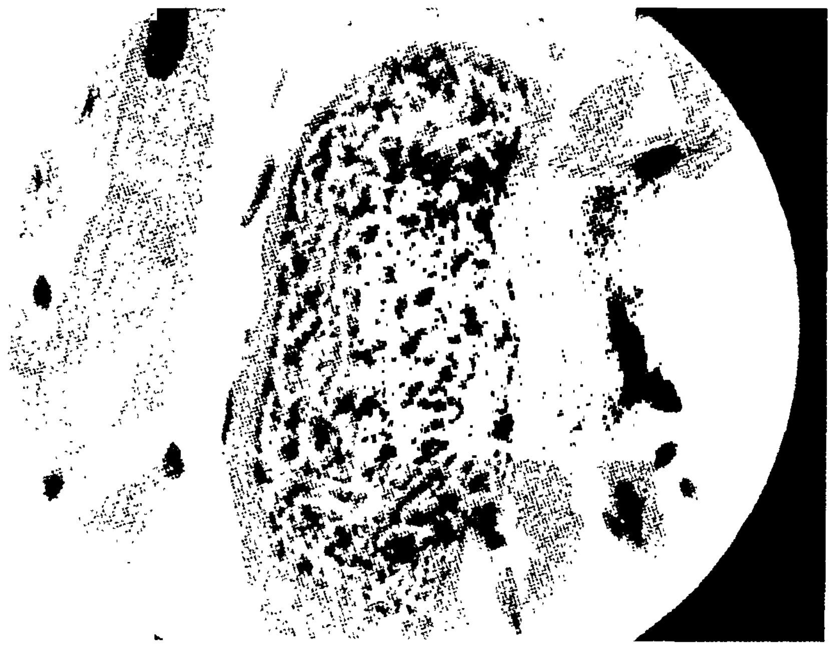
DESCRIPTION OF PLATES

PLATE 80

FIG. 1. Cyst in human cardiac muscle. (Patient A. W.) $\times$ 850.

FIG. 2. Cyst in thigh muscle of guinea-pig. Resemblance to human parasite in Figure 1 may be noted. $\times$ 850.

1



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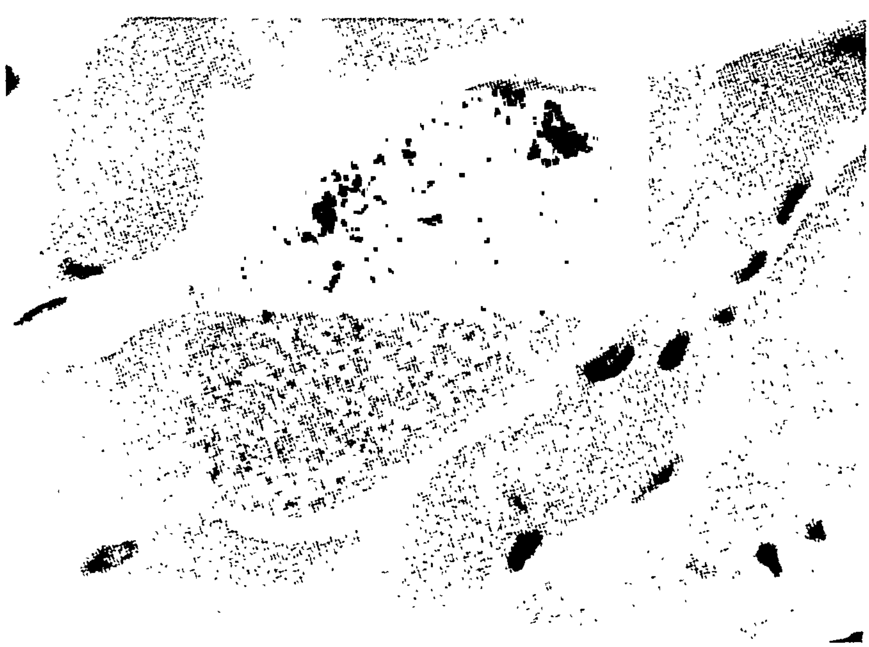


Kean and Grocott

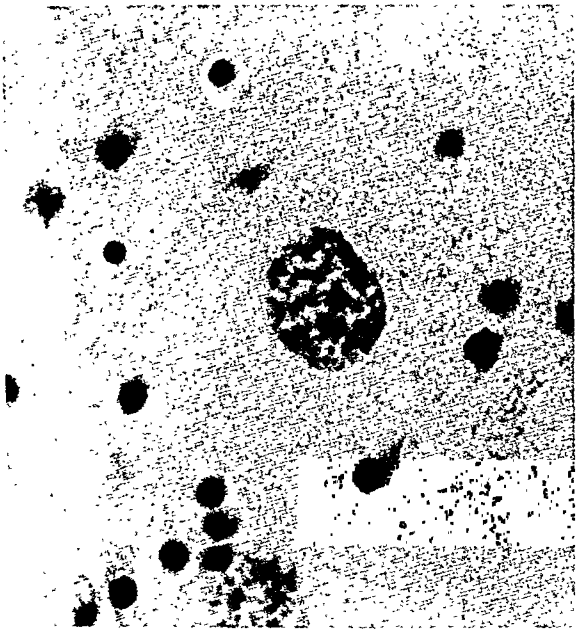
Sarcosporidiosis or Toxoplasmosis

PLATE 81

FIG. 3. Cyst or pseudocyst resembling Toxoplasma in brain of guinea-pig. A second cyst (out of focus) is present at the lower border of the field. × 850.

FIG. 4. *Sarcocystis tenella.* Parasite in heart of sheep, showing large cyst and individual sporozoites. Internal septa and a well defined capsule can be seen. × 850.

FIG. 5. *Sarcocystis miescheriana.* The cyst in cardiac muscle of a pig (not guinea-pig) has a thick striated capsule. × 850.



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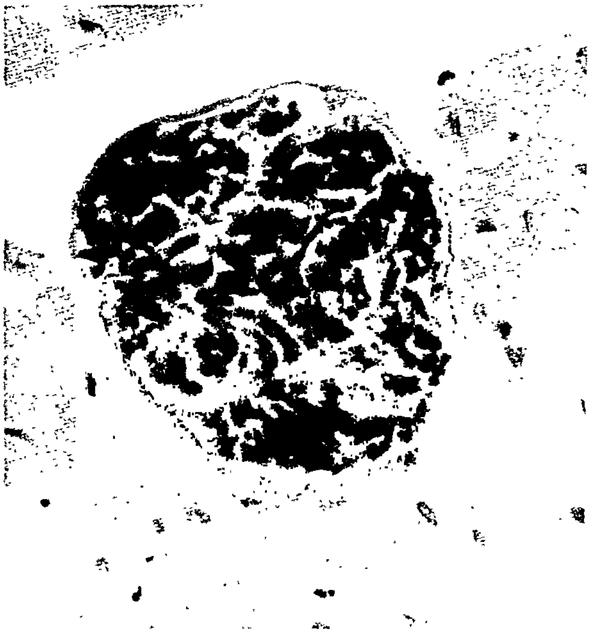

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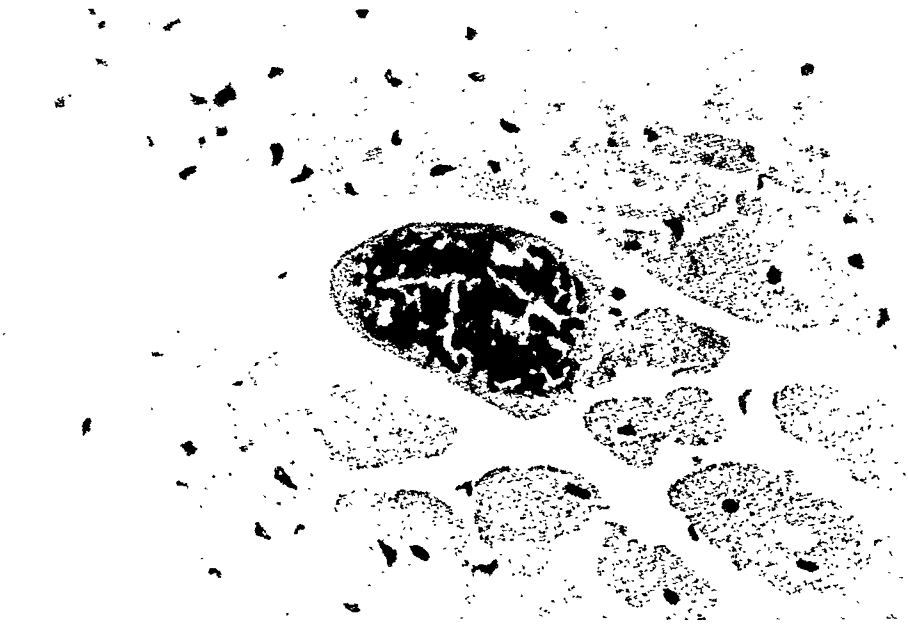

5

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Sarcosporidiosis or Toxoplasmosis

HEALED OR ARRESTED PULMONARY COCCIDIOIDOMYCOSIS

Correlation of Coccidioidin Skin Tests with Autopsy Findings *

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The renaissance of our knowledge of infections due to *Coccidioides immitis* occurred in 1937 with the description by Dickson,[1] Dickson and Gifford,[2] and Gifford, Buss, and Douds [3] of a widespread, benign, pulmonary infection in the San Joaquin Valley of California. Prior to that time the general concept of coccidioidal granuloma as a disseminated granulomatous disease with a 50 per cent mortality held sway. The demonstration that the minor respiratory illness often associated with erythema nodosum and commonly called "Valley Fever" was due to *C. immitis* stimulated widespread epidemiological study. Added clinical interest was aroused by the variety of pulmonary manifestations reported in early coccidioidomycosis, to wit: Bronchial pneumonia, subacute and chronic cavitation resembling tuberculosis, and calcified pulmonary lesions. The relationship of healed and calcified pulmonary lesions to positive coccidioidin skin tests forms the basis of this report.

Skin testing of patients for sensitivity to coccidioidin as a diagnostic measure or in epidemiological surveys has demonstrated a surprisingly high incidence of positive cutaneous tests depending largely on the locality of the study. The main contributors to our knowledge of this work have been Gifford, Buss, and Douds,[3] and Smith [4] in California. Farness [5] collected a series of such tests which ranged from 90 per cent positives in school children of the Pima Indian Reservation of Arizona and 58.2 per cent positives in school children of Kern County, California, to no positive reactions among school children of Philadelphia, New York, and Ann Arbor. A small series of adults in these eastern centers showed 1 to 5.9 per cent positives. The recent interesting experiences of the Medical Corps of the Army in California and Arizona have substantiated and added to the work of the earlier investigators.[6, 7]

The observations of Aronson and Gallagher [8] on Pima Indian children revealed such a high incidence of positive cutaneous reactions that the material was first thought to be nonspecific. However, the same antigen when used in testing Philadelphia children gave no positive reactions. Of greater interest was the fact that roentgen studies of the

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Indian youngsters showed calcified hilar lymph nodes, usually associated with tuberculous infections. Of extreme interest to epidemiologists is the isolation of *Haplosporangium parvum* from rodents in this region by Emmons.[9] This organism, according to the author, is "genetically related to *C. immitis* and in some manner to coccidioidomycosis."

Quiescent and calcified pulmonary lesions of coccidioidomycosis have been described on a number of occasions. A report of such lesions and a summary of the literature are found in a paper by Cox and Smith.[10]

The clinical material at the Santa Fe Coast Lines Hospital in Los Angeles is particularly suited for a study of the type undertaken. Patients admitted here are largely employees and their families. As such they come to the hospital from Arizona, New Mexico, and California. Northern California furnishes no patients, but the San Joaquin Valley, the Bay region about San Francisco, and all of Southern California provide a great number. As members of the hospital association, these patients return year after year for follow-up or for care of new illnesses. Many are observed over a period of 25 or 30 years. By routine testing of all hospital patients, we expected eventually to see in their final illness and to examine at autopsy a certain number of those upon whom skin tests had been done.

Methods

The coccidioidin used for the skin tests was obtained from C. E. Smith of Stanford University School of Medicine who has prepared the antigen for a number of epidemiological surveys. The method of its preparation is given in the paper by Aronson and Gallagher.[8]

For routine skin tests, 0.1 cc. of a 1:100 dilution of coccidioidin was used. The tests were read, together with the controls, at 24 and 48 hours. An area of erythema of 1 cm. or more was accepted as a positive reaction. For the tuberculin skin tests a purified protein derivative was employed.

Antigens listed in Tables II and III were prepared in the same manner as coccidioidin.

The lungs of autopsied cases were removed intact for roentgen ray studies. In this way calcified and fibrotic areas were localized and then excised from the lungs and peribronchial lymph nodes. One-half of the excised material was finely divided by grinding in a mortar with alundum. The remainder was decalcified and sectioned. The finely divided material, after standing in 0.05 per cent copper sulphate solution about 18 hours, was injected into the testicles of guinea-pigs. In some in-

stances it was impossible to follow this procedure as the lungs had been fixed in formalin prior to examination.

Results

Coccidioidin skin tests were performed on a total of 1,165 patients. Of these, 302, or 25.9 per cent, were positive reactors. Table I shows the geographical distribution and the results of the skin tests. It is evident that there are three major areas which are sources of infection in our material. The incidence of infection in these areas was as follows: The San Joaquin Valley, 62.8 per cent; Arizona, 28 per cent; and Texas, 35.7 per cent.

Attention is directed to the rather high percentage of positive reactors from the San Francisco Bay area. This figure is quite out of proportion to the known geographical distribution of acute coccidioidomycosis and is probably due to infections contracted in the San Joaquin Valley while on runs between San Francisco and the southern end of the Valley. The low incidence of coccidioidin positivity in Southern California is indeed quite surprising. However, the Santa Fe Railroad has not had a direct line between Los Angeles and San Francisco through the San Joaquin Valley until recent years. Moreover, we have known of only one case of coccidioidomycosis occurring in the vicinity of Los Angeles until the publication of the recent experiences of the Army. Except for these two discrepancies, our figures of coccidioidin positivity agree rather well with the known distribution of endemic areas of coccidioidomycosis.

Table I
Coccidioidin and Tuberculin Skin Tests

	Coccidioidin			Tuberculin (Purified protein derivative no. 2)			Tuberculin (Purified protein derivative no. 1)		
	Total cases	Positive	Per cent	Total cases	Positive	Per cent	Total cases	Positive	Per cent
San Joaquin Valley	156	98	62.8	27	22	81.4	20	3	15.0
San Francisco and Bay region	70	23	32.9	15	12	80.0	15	2	13.3
Arizona	250	70	28.0	38	29	76.3	30	4	13.3
Los Angeles and Southern California	615	99	16.1	100	80	80.0	134	20	14.9
New Mexico	60	7	11.6	12	10	83.3	9	1	11.1
Texas	14	5	35.7	1	1				
Total	1,165	302	25.9	193	154	79.7	208	30	14.4

Thirty-six patients upon whom skin tests had been performed were examined by autopsy. Twenty-five had reacted negatively to the coccidioidin skin test. Four of the negative cases showed no calcified lesions of the lungs. The lungs of 3 of the 25 had small calcified areas throughout the lung fields, suggestive of a healed miliary type of tuberculosis. The roentgenograms of the remainder of the negative reactors revealed calcified areas of different sizes in various parts of the lungs and in moderate numbers. In no instance was a single calcified lesion found. However, in most instances, one of the calcified areas was found to be larger than the remainder in the individual case. These areas of calcification varied from 4 to 20 in number and from 1 to 10 mm. in diameter. There was no uniformity of location except that the lesions were close to the walls of the bronchi or bronchioles and in peribronchial lymph nodes. In all probability most of these lesions were of tuberculous origin and were residual evidences of healed primary foci. All of the negative coccidioidin reactors had apical pleural scars except the 3 without post-mortem roentgenographic evidence of pulmonary calcification. These 3 cases constitute 12 per cent of the negative reactors. Of considerable interest is the fact that these three individuals were tuberculin negative.

The age limits of the negative coccidioidin reactors were from 38 to 82, with 19 of the 25 patients past 60 years.

Material from 7 of the negative reactors was injected into guinea-pigs. Material from 20 cases was cultured on two types of media. The animals and cultures were negative for *Mycobacterium tuberculosis*. This would indicate that calcified lesions in the higher age brackets are sterile regardless of their cause.

Spherules suggestive of *C. immitis* were found in the calcified lesions of one of the negative coccidioidin skin reactors. However, careful search of the histological material failed to reveal endosporulation essential to the differentiation of coccidioides from organisms of similar morphology known and unknown.

Two of the negative coccidioidin reactors were excluded from the negative series as both patients died of coccidioidal granuloma. One had typical Addison's disease with coccidioidal involvement of the adrenals. The other succumbed to a diffuse pulmonary involvement. These cases are examples of the well known fact that patients with progressive coccidioidal granuloma not infrequently react negatively to coccidioidin. Cultures and animal inoculations were positive in each case for *C. immitis*. The interesting feature of each case relevant to the subject of this paper was the finding of primary foci in the lungs and peribronchial lymph nodes of older duration than the advancing pulmonary lesions.

Case Histories of the Positive Coccidioidin Skin Reactors Seen at Autopsy

Case 1

A boy, age 15, from Bakersfield, California, entered the Santa Fe Hospital in December, 1939, complaining of chills, fever, loss of weight, and enlarging abdomen. These symptoms had been manifested periodically from August 1, 1939. He had a moderate anemia and a leukocytosis of 27,000 with 80 per cent polynuclear leukocytes. The coccidioidin skin test was markedly positive. At autopsy, a malignant hepatoma was found with metastases to the lungs. Three cm. below the right apex, close to the pleura, there was a small, fibrotic, encapsulated, calcified area of caseation 1.5 cm. in diameter. A smaller area of calcification was found in a peribronchial lymph node on the right side. Animal inoculations were not performed, but cultures were positive. Inoculations of the fungus culture into guinea-pigs failed to produce lesions. Sections of the lesions revealed a caseous area enveloped by a thick, fibrous-tissue capsule in which there was finely divided calcified material. Spherules and endospores were found. The lesions in the lymph node consisted almost entirely of fibrous tissue in which there was carbon pigment, calcium, and spherules of *C. immitis*.

Case 2

A white male, age 60, a section foreman, from Reedly, California, died on June 18, 1942, of arteriosclerotic and hypertensive heart disease. His coccidioidin skin test had been strongly positive on a previous hospital entry. Gross examination of the lungs revealed the presence of apical scars and many small areas of calcification in the tracheobronchial lymph nodes. In the upper anterior part of the left lower lobe there was an area of encapsulated caseation that had a diameter of 3 mm. The lesions were removed, sectioned, and injected into the testicles of guinea-pigs. One pig, injected with lesions from the lung, developed a testicular abscess which was negative for *C. immitis* and *Myco. tuberculosis*. Histological preparations of the pulmonary lesions revealed the presence of spherules but no endospores.

Case 3

A white male, age 65, a section foreman, from San Bernardino, California, died in the Santa Fe Hospital on January 29, 1941. Death was due to carcinoma of the prostate, osteosclerotic anemia, and terminal bronchopneumonia. Post-mortem roentgenograms of the lungs revealed areas of calcification in the peribronchial lymph nodes and multiple very small areas of calcification in both lower lobes. A larger area of encapsulated caseation with calcification was found in the upper anterior portion of the lower right lobe. Microscopical examination of the caseous area from the lung revealed the presence of many spores, shells of spores and a rare spherule filled with endospores. The lesion was typical of the healed lesion to be described. Areas of fibrosis were found in the peribronchial lymph nodes, but no *C. immitis* were demonstrated. Cultures from the primary lesion were negative. Animal inoculation was not made.

Case 4

A white male, age 60, a towerman, entered the hospital on January 22, 1942, and died on March 11, 1942. This patient was from Berkeley, California. Coccidioidin skin test was strongly positive. The cause of death was bronchopneumonia complicating carcinoma of the lung. Post-mortem roentgenograms of the lungs revealed fine areas of calcification and caseation, less than 1 cm. in diameter, in the lower right lobe, and one similar area of calcification in a peribronchial lymph

node on the right side. Representative areas were sectioned and ground up for cultures and animal inoculations. The sections showed areas of walled-off caseation in which there was little calcification. No spores of coccidioides were found and animal inoculations and cultures were negative for tubercle bacilli and *C. immitis.*

Case 5

A white American male, age 64, whose home was in Los Angeles, died in the Santa Fe Hospital of congestive heart failure resulting from hypertensive and arteriosclerotic heart disease. A coccidioides skin test was reported as weakly positive. The area of hyperemia was less than 1 cm. in diameter. A tuberculin skin test was negative. The autopsy revealed apical scars and pleural adhesions. Post-mortem roentgenograms of the lungs showed minute areas of calcification about 1 mm. in diameter in the lower part of the lower right lobe, a few similar areas in the upper part of the upper right lobe, and one such area in the left lower lobe towards the periphery. Two larger areas of calcification were found along one of the first branches of the right main bronchus. Two were confined to peribronchial lymph nodes. Microscopical examination of these areas revealed the presence of areas of caseation, silicotic nodules, and much fibrosis. Cultures and animal inoculations were negative for tubercle bacilli and *C. immitis.* The lesions were most likely of tuberculous origin.

Case 6

A white American male, age 63, a tailor, died on February 3, 1941, in the Santa Fe Hospital of complications resulting from carcinoma of the stomach. No skin tests had been performed. In the lower left lung, centrally located, there was a large area of encapsulated caseation. No apical scars or pleural fibrosis were noted. The area of caseation was surrounded by a thick, fibrous capsule in which there were a moderate number of round cells. Scattered throughout the caseous material at all levels there were large numbers of spores varying in size. In addition there were many large spores filled with endospores. Unfortunately, cultures and animal inoculations were not done. This is an example of a typical arrested pulmonary lesion of coccidioides. Twenty-five years ago this patient lived in Taft, California, for a period of several years. During the past few years he had returned to Taft for short visits. There was no history of upper respiratory infection or attacks of influenza in the years immediately preceding his death.

Case 7

The patient was a white male, age 60, whose home was in Gallup, New Mexico. Death was due to right cardiac failure, emphysema and chronic bronchitis. The lungs were placed in formalin before examination; therefore, no culture or animal inoculation was possible. Gross examination of the lungs revealed apical scars and thickening of the pleura. Post-mortem roentgenograms of the lungs showed three areas of calcification in the peribronchial lymph nodes on the left side, each about 1 cm. in diameter. In the base of the left lung there was a small area of calcification. These areas were excised and studied. In the section of the pulmonary lesion, which consisted of an oval area of caseation surrounded by a thick capsule, there were many oval bodies having central dense areas. No spherules were found in the lesions of the peribronchial lymph nodes.

Case 8

An American Negro, age 69, from Bakersfield, California, died in the Santa Fe Hospital of carcinoma of the stomach. The coccidioidin skin test was positive while the tuberculin skin test was negative. Five oval areas of calcification were found in the lower portion of the left lung and two similar areas in the upper right lobe. In a peribronchial lymph node on the left there was a rather large area of calcifica-

tion. Animal inoculations and cultures of the post-mortem material were negative. Sections of the calcified areas from the left lung contained an area of necrosis surrounded by a fibrous tissue capsule. A few spherules and a rare shrunken spore showing endosporulation were found.

Case 9

An American white male, age 65, a switchman, from Barstow, California, died in the Santa Fe Hospital of syphilis of the aorta and congestive heart failure. The coccidioidin skin test was positive. The tuberculin test was negative. Post-mortem roentgenograms revealed five areas of calcification in each lung. The largest had a diameter of 6 mm. Animal inoculations and cultures were negative for *C. immitis* and *Myco. tuberculosis.* Spherules, but no endospores, were found in one of the lesions of the lungs.

Case 10

A Negro male, age 54, a porter, whose home was in Los Angeles, died in the Santa Fe Hospital of congestive heart failure due to hypertensive heart disease. Coccidioidin skin test was positive. Post-mortem roentgenograms of the lungs showed one area of calcification at the base of the right lung, 0.5 cm. in diameter, and one at the base of the left lung, 0.3 cm. in diameter. A peribronchial lymph node in the left side contained a small area of calcification. Apical scars were not present. Spherules, but no endospores, were found in the pulmonary lesions. Animal inoculations and cultures were negative for *C. immitis* and *Myco. tuberculosis.*

Case 11

The patient was a white male, age 64, who lived in Bakersfield, California. Death was due to carcinoma of the tongue and terminal bronchopneumonia. Autopsy revealed obliteration of the pleural cavities by old fibrous adhesions. No apical scars were noted. Areas of calcification were found in the lower right lobe laterally and superiorly. Four peribronchial lymph nodes contained areas of calcification. Cultures and animal inoculation were negative for *Myco. tuberculosis* and *C. immitis.* No spores or endospores were found in the histological preparations.

Discussion

Dickson and others, on a number of occasions, have called attention to the similarity of coccidioidomycosis and tuberculosis. It is remarkable how this similarity prevails in the interpretation and reading of the skin tests and the appearances of the healed pulmonary lesions. As with the tuberculin test and tuberculosis, there is a rough quantitative relationship between the coccidioidin skin reaction and the activity or age of the primary coccidioides lesions. Moreover, a negative coccidioidin skin test may be obtained in the late stages of coccidioidal granuloma. Two such examples are given in Table IV; one patient dying of disseminated pulmonary coccidioidal granuloma and the other of Addison's disease due to coccidioides infection of the adrenals.

It is probable that persons with healed lesions of coccidioidomycosis may eventually lose their skin sensitivity to coccidioidin. One of the negative skin reactors had a calcified pulmonary lesion that contained spherules but no endospores.

There is no apparent cross-antigenic relationship of tuberculin and coccidioidin. This is apparent in Table II. Furthermore, a breakdown of 187 positive reactors to tuberculin (purified protein derivative, second strength) into geographic locations, as shown in Table I, shows no appreciable variation, whereas the positive reactors to coccidioidin are definitely localized to super-endemic areas.

The question of nonspecificity of coccidioidin was further studied by simultaneous skin tests of coccidioidin and antigens of other fungi. Antigens of Blastomyces, Aspergillus and Sporotrichum were so tested with completely negative results, as shown in Tables II and III. During the course of these investigations, a patient with blastomycosis of the scrotum was admitted to the service. He reacted negatively to coccidioidin in dilution of 1:1000, 1:100 and 1:10. Unfortunately, the patient left the hospital before we were able to have a blastomyces antigen prepared.

TABLE II

Comparison of Coccidioidin and Tuberculin Skin Tests

Coccidioidin	Tuberculin	Total cases	Per cent
Positive	Positive	60	8.5
Negative	Positive	158	22.5
Positive	Negative	133	19.0
Negative	Negative	349	49.8
Total		700	

No clear clinical histories suggestive of coccidioidomycosis infections could be obtained from the positive reactors. This would suggest that subclinical infections are more common than the acute clinical phase of coccidioidomycosis. However, there are undoubtedly many unrecognized cases of coccidioidomycosis.

Calcified lesions of coccidioidomycosis were indistinguishable grossly from similar lesions of tuberculosis except that apical scars were not a part of the healed or arrested pulmonary phases of coccidioides. The primary disease resolves into one or more encapsulated fibrotic areas in various parts of the lung with associated single or multiple similar lesions in the peribronchial and tracheobronchial lymph nodes. These lesions in some cases were so small as not to be revealed on the roentgenograms taken during life. Furthermore, such lesions were easily missed unless roentgenograms were made of the lungs after removal from the thoracic cage. From examination of such films it becomes apparent that a single healed pulmonary lesion was a rarity. No doubt primary tuberculosis also resolves in multiple, rather than single, healed lesions of the parenchyma of the lungs.

The histological pattern was essentially that of tuberculosis except for the presence of the spherules. The centers were caseous and contained small calcified particles and faint outlines of necrotic lung tissue. Each lesion was surrounded by a dense, hyalinized, fibrous-tissue capsule, external to which there were collections of lymphocytes. In the older lesions the caseous material may be replaced by fibrous tissue. Located in the capsules and caseous material there were spherules of *C. immitis* and fewer large spherules containing endospores. Many of the organisms were shrunken, distorted, or ruptured. Varying amounts of calcification were noted in some of the spherules.

TABLE III

Comparison of Skin Tests with Coccidioidin and with Preparations of Other Pathogenic Fungi

Cases	Coccidioidin	Torula	Aspergillus
6	Positive	Negative	Negative
9	Negative	Negative	Negative

Cases	Coccidioidin	Blastomyces	Sporotrichum
8	Positive	Negative	Negative
18	Negative	Negative	Negative

Spherules were present in the healed lesions of 8 of the positive reactors. However, endosporulation was noted in only 5 of the 8 cases. We consider endosporulation essential in establishing the lesion as one of coccidioides. Inasmuch as budding was not demonstrated in any of these cases, we were inclined to accept the presence of spherules as presumptive evidence of healed coccidioidomycosis until the discovery by Emmons [9] of *Haplosporangium parvum*. However, 45.4 per cent of the 11 positive reactors seen at autopsy had proved healed lesions of coccidioidomycosis. Certainly this is a considerably higher figure than obtains in similar studies of the healed lesions of tuberculosis.

Considerable significance is attached to the fact that in none of the animals injected with material from the healed lesions was a lesion of coccidioides or tuberculosis demonstrated. Undoubtedly most of the healed lesions were of tuberculous origin. This would indicate that in healed tuberculosis, as well as healed coccidioidomycosis, the organisms are dead. Therefore, after a certain period it is unlikely that these lesions could be responsible for the disseminated and fatal forms of either disease. Moreover, the two fatal cases of coccidioidal granuloma (nos. 12 and 13, Table IV) were examples of slowly advancing, primary pulmonary forms with evidence of healing in the initial lesion.

TABLE IV

Positive Coccidioidin Skin Reactors: Cases Examined by Autopsy

Sex	Age	Skin reaction		Cause of death	Case no.	Guinea-pig inoculation	Cultures		Tissue findings	
		Coccidioidin	Tuberculin				*Coccidioides immitis*	Tubercle bacilli	Endospores	Spherules
M	15	Pos.	Neg.	Carcinoma, liver	1		Pos.	Neg.	Pos.	Pos.
M	60	Pos.		Heart disease	2	Neg.			Neg.	Pos.
M	65	Pos.		Carcinoma, prostate	3		Neg.	Neg.	Pos.	Pos.
M	60	Pos.		Carcinoma, lung	4	Neg.	Neg.	Neg.	Neg.	Neg.
M	64	Pos.	Neg.	Heart disease	5	Neg.	Neg.	Neg.	Neg.	Neg.
M	63	Pos.		Carcinoma, stomach	6				Pos.	Pos.
M	60	Pos.		Heart disease	7				Pos.	Pos.
M	69	Pos.	Neg.	Carcinoma, stomach	8	Neg.	Neg.	Neg.	Pos.	Pos.
M	65	Pos.	Neg.	Heart disease; syphilis	9	Neg.	Neg.	Neg.	Neg.	Pos.
M	54	Pos.	Neg.	Heart disease	10	Neg.	Neg.	Neg.	Neg.	Pos.
M	64	Pos.	Neg.	Carcinoma, tongue and prostate	11	Neg.	Neg.	Neg.	Neg.	Neg.
M	45	Neg.	Neg.	Coccidioidal granuloma, pulmonary	12	Pos.	Pos.	Neg.	Pos.	Pos.
M	60	Neg.	Neg.	Addison's disease, due to coccidioidal granuloma of adrenals	13	Pos.	Pos.	Neg.	Pos.	Pos.

Blank spaces = tests not performed.

Conclusions

1. The incidence of positive coccidioidin skin tests in the Santa Fe Coast Lines Hospital is 25.9 per cent.
2. No cross-antigenic relationship was demonstrated between tuberculin and coccidioidin.
3. Skin antigens of other fungi prepared in a manner similar to that for coccidioidin produced no skin reactions in patients reacting positively to coccidioidin.
4. A case of cutaneous blastomycosis reacted negatively to the coccidioidin in several dilutions.
5. As to its clinical significance, the coccidioidin skin test is to be evaluated in the same manner as the tuberculin skin test.
6. Autopsies revealed healed calcified lesions in the lungs of all positive coccidioidin skin reactors. Grossly, the healed primary pulmonary lesions of coccidioidomycosis are indistinguishable from similar lesions of tuberculosis.
7. Spherules and endospores of *C. immitis* were found in 45.4 per cent of the positive reactors. Spherules, but no endospores, were found in three additional cases of the positive coccidioidin group.

We are greatly indebted to the internes of the Santa Fe Hospital, and particularly to Dr. Otto Lange, for their help in performing the skin tests. We are extremely appreciative of the aid extended by Dr. Charles E. Smith of Stanford University School of Medicine.

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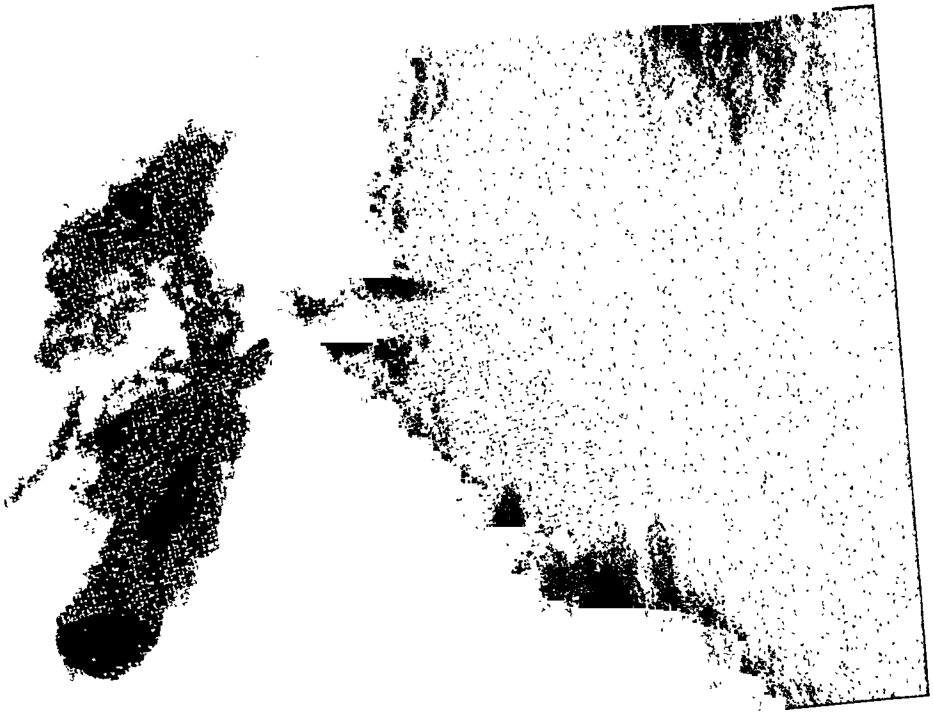
DESCRIPTION OF PLATES

Plate 82

Fig. 1. Case 1. Post-mortem roentgenogram of lungs. Area of calcification in upper right lobe, healed coccidioidomycosis. Smaller areas of calcification in right peribronchial lymph nodes. Other shadows are due to metastatic malignant hepatoma.

Fig. 2. Case 1. Lesion found in right upper lobe. × 20.

Fig. 3. Case 1. Peribronchial lymph node with spherules present in the center of an area of fibrosis. × 100.



1

2

3

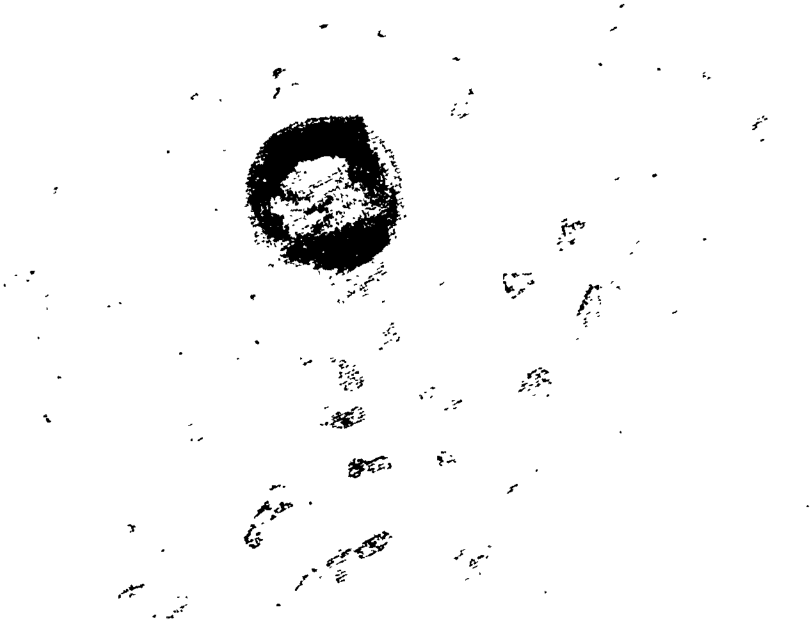
Pulmonary Coccidioidomycosis

Butt and Hoffman

PLATE 83

FIG. 4. Case 1. Shrunken, partly calcified spherule with endosporulation. × 1200.

FIG. 5. Case 1. Spherules of *Coccidioides immitis* in a peribronchial lymph node. × 1200.



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Pulmonary Coccidioidomycosis

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PLATE 84

FIG. 6. Case 3. Area of calcification in hilar region of right lung. Smaller areas of calcification in lower left lobe and peribronchial lymph nodes.

FIG. 7. Case 3. Lesion in hilar region of right lung. $\times$ 100.

FIG. 8. Case 3. Lesion of right lung showing spherules and spherules with endospores. $\times$ 450.

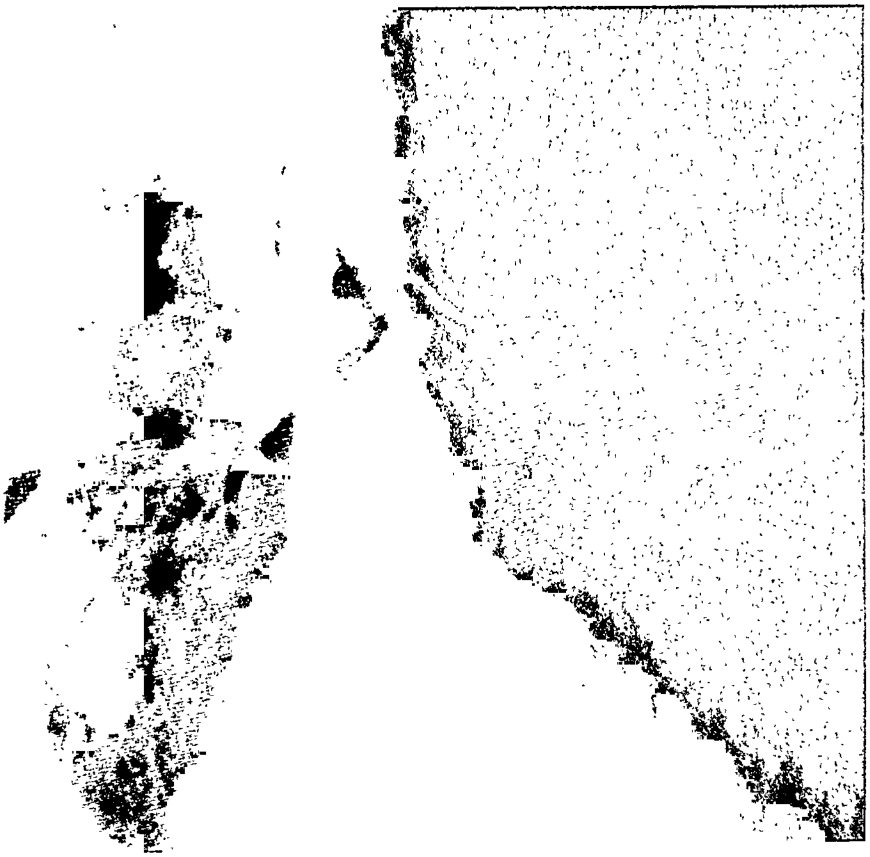

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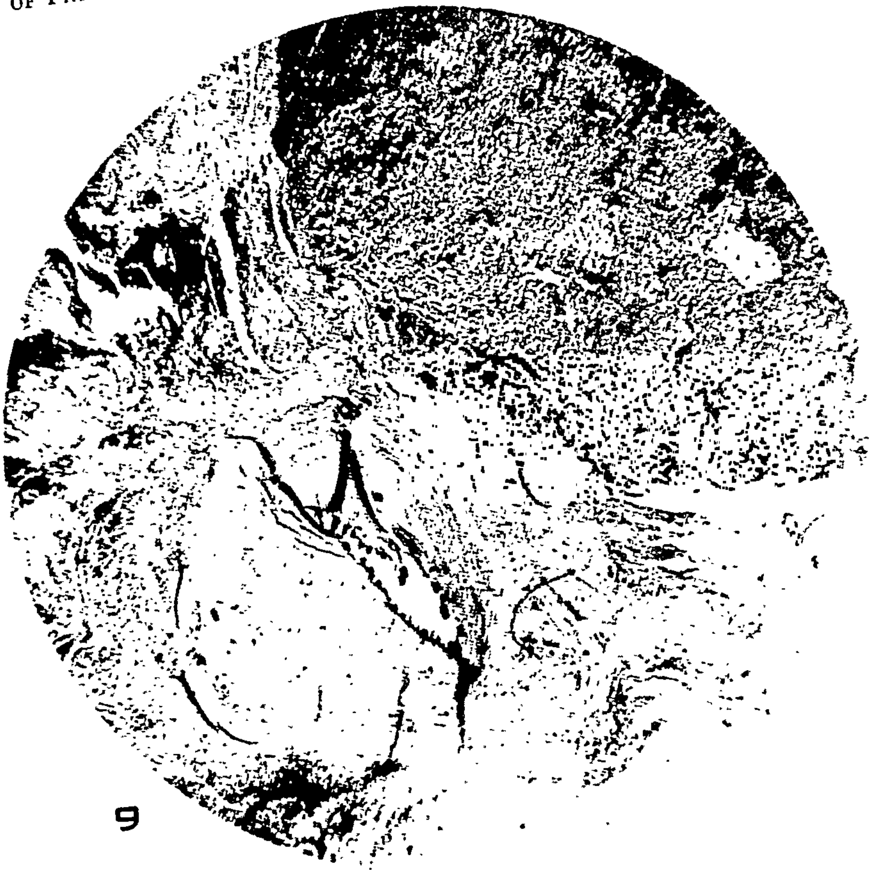
PLATE 85

FIG. 9. Case 3. Peribronchial lymph node showing fibrotic oval mass containing shrunken pink-staining spores and carbon pigment. × 22.

FIG. 10. Case 6. Deformed spherule of *Coccidioides immitis* with endospores. × 1080.



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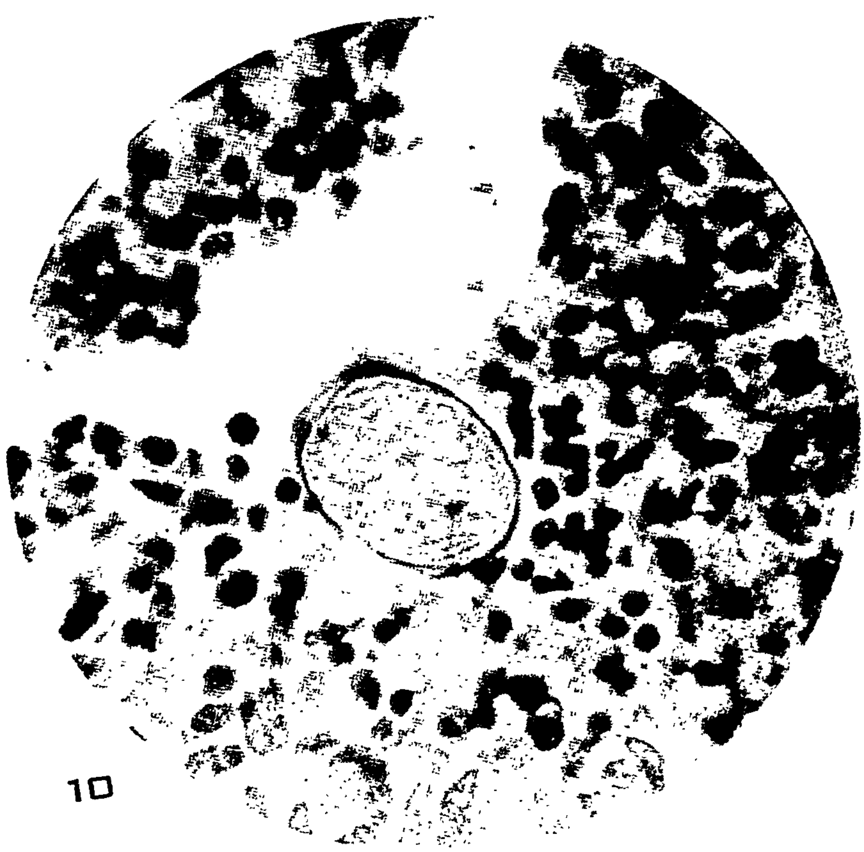

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Pulmonary Coccidioidomycosis

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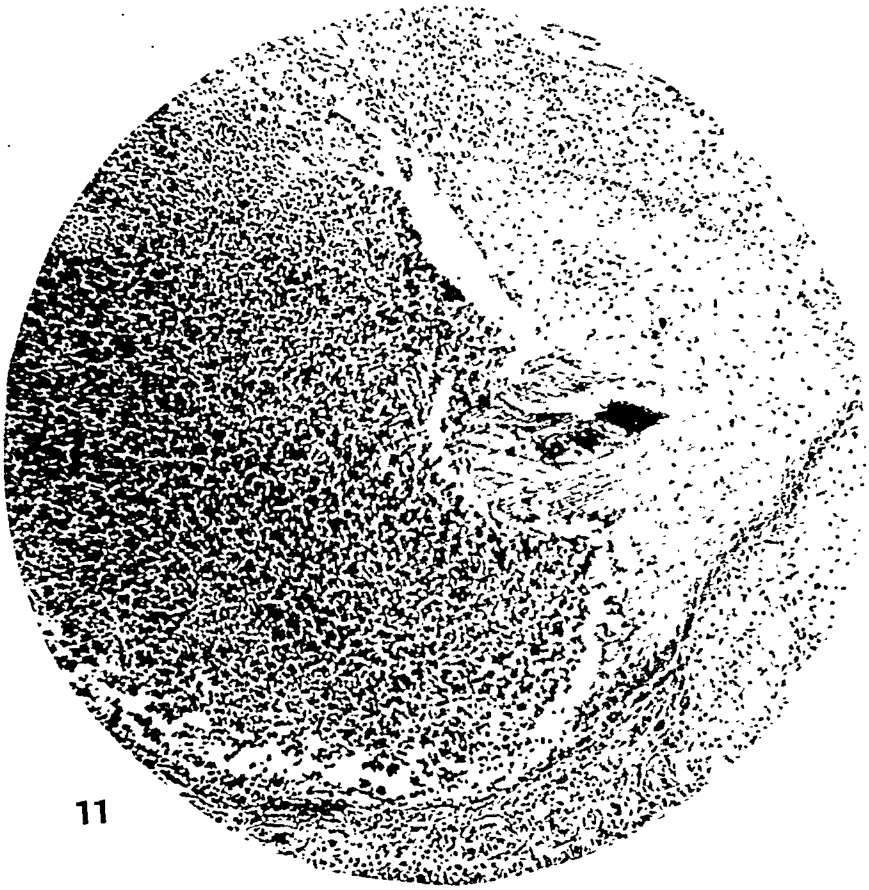
PLATE 86

FIG. 11. Cutaneous blastomycosis. Coccidioidin skin test was negative. × 90.

FIG. 12. Cutaneous blastomycosis showing budding organisms. × 1080.



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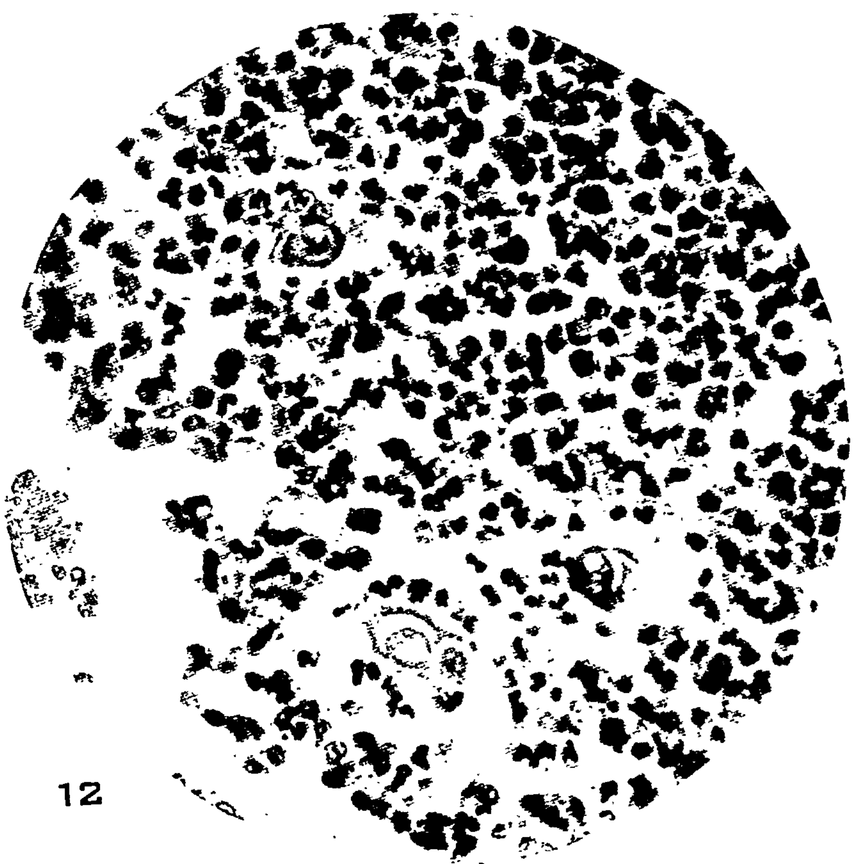

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Pulmonary Coccidioidomycosis

SUBACUTE BACTERIAL (STREPTOCOCCUS VIRIDANS) PULMONARY ENDARTERITIS *

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Clinical cases in which *Streptococcus viridans* has produced vegetations on the intima of the pulmonary artery are infrequent. Such lesions have been encountered under various conditions.

Vegetations in subacute bacterial endocarditis characteristically extend from their original location on the valves to the surrounding mural endocardium. Similarly, this tendency results in spread from the pulmonary semilunar valves for some distance up the pulmonary artery. One may assume that this mechanism prevails in the cases presenting a vegetative lesion involving, in continuity, both the semilunar valves and the stem of the pulmonary artery. These cases occur comparatively frequently. They usually show vegetations also on the valves of the left side of the heart. Old rheumatic lesions are commonly present upon which the bacterial process is superimposed.

Isolated vegetations situated in the pulmonary artery above the valves are associated with pathological lesions of a varied nature. These lesions are thought to account for the unusual localization of the vegetations, and of them congenital defects in the heart and great vessels are the most important. Patent ductus arteriosus is the most common deformity in this group. Abbott [1] found that 21 of 92 patients with patent ductus arteriosus died with subacute bacterial endarteritis superimposed on the defect. The vegetations developed in the pulmonary artery adjacent to the ductus where the arterial wall is subjected to the constant impact of the blood which is forced from the aorta into the pulmonary artery. Other congenital lesions are occasionally associated with *Str. viridans* infection in the pulmonary artery. This is illustrated by a case with congenital interventricular septal defect, persistent conus arteriosus, and bicuspid pulmonary valve reported by Posey.[2]

A variety of pathological processes other than congenital deformities may also predispose to the nidation of *Str. viridans* in the pulmonary artery. These, however, are very infrequent. An example is the case reported by Marchal, Porge, and Ortholan [3] of an aortic aneurysm impinging on the pulmonary artery.

Mehlin's [4] first case of *Str. viridans* infection, in a man, 25 years old, presented a vegetation which was confined to the stem of the pul-

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monary artery. The wall of the vessel was otherwise normal. The pulmonary semilunar valves, however, were thickened and rigid, confirming the clinical diagnosis of pulmonary insufficiency which was made several years before death. The myocardium also contained numerous small scars. The lesions of the pulmonary cusps and the myocardium were apparently the result of rheumatic disease. Though the wall of the pulmonary artery was thought to be normal, except for the vegetation, one will recall the changes occurring with rheumatic fever in the pulmonary artery as described by Kugel and Epstein.[5] It may be suspected, therefore, that previous involvement, though unnoticed, was responsible for the localization of the vegetation in the pulmonary artery in Mehlin's first case.

The literature contains several reports of cases of isolated vegetations in the pulmonary artery that are impossible to evaluate since no bacteriological studies were made. Such instances are Mehlin's third case, the second case in Reiche's [6] series (1892), and Oberndorfer's [7] two cases (1918). The cases of Reiche and Oberndorfer were reviewed by Mehlin.[4] An analysis of the available data, however, makes it appear improbable that they were caused by *Str. viridans*.

In reviewing the literature, no instance of isolated vegetations in the pulmonary artery above the semilunar valves caused by *Str. viridans* has come to my attention in which both predisposing changes in the wall of the pulmonary artery and pathological changes in the heart and aorta were absent. However, this situation prevailed in the following case.

Report of Case

W. P., Unit no. 42433. A white girl, 4½ years old, was admitted on November 20, 1943, with the history of a prolonged fever.

Past History. The child had had occasional colds. She had been immunized against diphtheria.

Family History. The information obtained concerning the family history was noncontributory.

Present Illness. The child had been sick with fever and anorexia for about 7 weeks. Two weeks after the onset, the patient's left knee became tender but was not swollen. The tenderness persisted for 10 days, then the large joints of the right upper extremity became similarly involved. The patient had severe sweats and frequent epistaxis during the 2 weeks prior to admission. Antimalarial treatment and sulfa drugs were without benefit.

Physical Findings. The patient was acutely ill and poorly nourished. The skin and mucous membranes were pale. The rectal temperature was 104.4° F. The pulse was rapid and bounding. A cough was present and coarse râles were heard throughout the lungs. The apex beat of the heart was immediately lateral to the midclavicular line. A systolic murmur was heard in the pulmonic area and the second pulmonic sound was thought to be accentuated. The spleen was enlarged. The right wrist, elbow, and shoulder were tender but not swollen.

Laboratory Findings. The red blood cell count was 1.59 million per cmm. and the hemoglobin was 4½ gm. per cent. The white blood cells numbered 11,750 per

cmm., with the following differential count: juveniles, 2 per cent; stabs, 6 per cent; segmented polymorphonuclear leukocytes, 77 per cent; lymphocytes, 14 per cent; monocytes, 1 per cent. Urinalysis revealed 1 plus albumin; red blood cells and casts were not found. Several large areas of consolidation in the periphery of the lower portion of the right lung were demonstrated by a roentgenogram. Blood culture yielded *Str. viridans.*

Clinical Course. The patient grew progressively worse during the 7 days of hospitalization. Her cough increased in severity. The respirations were labored and their rate increased to 50 per minute. Her pulse rate was 160 per minute. The temperature was intermittent in type, ranging between 98° and 105° F. There was some bleeding from the rectum. Slight edema of the face and ankles was present on the day of death.

Autopsy Findings

The autopsy was performed 6 hours after death. The following significant pathological changes were present:

The *heart* presented small areas of fibrin on the epicardium. The pericardial sac contained 50 cc. of clear fluid. All of the chambers of the heart were dilated, those on the right more than those on the left side. All valves were delicate and translucent. The mural endocardium glistened. The chordae tendineae were grossly normal. The aorta appeared intact. Histological sections from the heart failed to reveal any significant pathological changes.

The *pulmonary artery* showed one large and several small vegetations (Fig. 1). The large vegetation arose with a broad base from the right side of the pulmonary artery at the level of its division into the right and left main branches. It measured 2 cm. in its widest diameter and extended for 1 cm. into the right main branch. An ulceration of the vessel wall could be seen only after removal of the firmly attached vegetation. The arterial wall was thickened at the site of the lesion. *Str. viridans* was cultured from this vegetation. Several vegetations measuring 2 to 3 mm. were found on the left side of the pulmonary artery. The most proximal of these was situated 2 cm. above the semilunar valves, while the most distal one was found in the left main branch 2.5 cm. above the point of division of the pulmonary artery. The lesions were separated from each other by normal appearing intima and vessel wall. The circumference of the pulmonary artery, determined 2 cm. above the semilunar valves, exceeded the circumference of the aorta at a corresponding level by 2 mm., which is a normal finding. No congenital malformation was found.

Sections were taken through both the large and the small vegetations. Since the latter lesions represented a less advanced stage of involvement, their histological appearance is described first. They consisted almost entirely of gram-positive cocci, often distinctly arranged in chains. Weigert's stain revealed a destruction of the elastic lamellae

at the base of these vegetations (Fig. 4). This change was most severe near the bacteria, concentrically decreasing in severity toward the periphery. Faintly staining remnants of elastic tissue were present within the vegetation. The destruction of the inner layers of the arterial wall was accompanied by a considerable inflammatory reaction (Fig. 2). The inflammatory cells extended into the adventitia. Polymorphonuclear leukocytes were more numerous, though not abundant, in the vicinity of the vegetation while in the more distal areas lymphocytes and macrophages predominated. Numerous fibroblasts and capillaries were present at the base of the vegetation. The vasa vasorum did not reveal any significant changes.

Microscopical sections through the large vegetation showed extensive destruction of the underlying arterial wall as illustrated by the elastic tissue stain. The infiltration with inflammatory cells was similar in location to, but more extensive than, that encountered in the small vegetation. The fibroblastic proliferation was conspicuous at the base of the vegetation, where occasional giant cells also were found (Fig. 3). An attempt at organization of the vegetation was noted. Bacteria were few in number and they were confined to the area of the vegetation near the lumen. In addition to the diffuse inflammatory infiltration, focal accumulations of inflammatory cells were seen in the deeper layers of the wall.

The *right lung* showed numerous large, dark red, raised, firm, wedge-shaped areas in the periphery of the lower and middle lobes. Central softening of these lesions with the formation of a small amount of purulent material was noticed in two instances. Thrombi or emboli were present in the blood vessels near the apices of some of the infarcts. A patchy consolidation of the remainder of the right lower lobe was found. Numerous bronchioles contained purulent exudate. Microscopical sections from the right lung revealed areas where all the alveoli were filled with red blood cells. Necrosis of infarcts was observed but the areas of necrosis were largely walled off by great numbers of fibroblasts growing out with abundant capillaries. The paucity of leukocytes in the necrotic focus as well as in the surrounding tissues was conspicuous. The *left lung* failed to reveal any infarcts. There were patchy areas of consolidation in the dependent portions and a purulent exudate was seen in numerous bronchioles. Groups of alveoli and bronchioles filled with inflammatory exudate were found in sections from both lungs.

The *spleen* weighed 218 gm. It was moderately soft and the follicles were swollen. Microscopically, the splenic pulp was hyperplastic. The *liver* was enlarged, grayish, and friable. The microscopical sections

revealed small fat globules in the liver cells and an infiltration of lymphocytes about the portal spaces. The *kidneys* were pale and flabby. Histologically, there was some cloudy swelling of the epithelial cells in the tubules. The glomeruli were intact.

Pathological-Anatomical Diagnoses. Subacute bacterial (*Str. viridans*) pulmonary endarteritis; serofibrinous pericarditis; multiple septic infarcts in right lung; moderate hyperplasia of the spleen; parenchymatous degeneration of the liver and kidneys; bronchopneumonia, bilateral.

Discussion

It may be thought that, upon closing, the ductus arteriosus had left a defect in the arterial wall on which the vegetation became superimposed. This supposition was untenable, however, since the large vegetation arose from the right side of the stem of the pulmonary artery and from the adjacent part of the right main branch. The ductus arteriosus links either the left circumference of the stem of the pulmonary artery or its left main branch with the aorta.

Allen [8] analyzed the nature of the vegetations in bacterial endocarditis with the aid of differential connective tissue stains. He observed that the bulk of the vegetations, protruding into the lumen, was formed by necrotic tissue derived from the involved valve. Thrombotic material contributed to the formation of the vegetation to a much smaller extent, according to Allen. The histological structure of the vegetations in the present case confirms Allen's observations, which seem to apply to mural vegetations as well as to those on valves. Elastic tissue stains from a small vegetation revealed that a large part of the projecting mass was composed of tissue derived from the arterial wall, as demonstrated by the faintly staining remnants of elastic tissue in the amorphous material (Fig. 4). It is suggested that marked localized swelling of the part of the wall exposed to the bacterial action causes it to bulge into the lumen. After this tissue has become necrotic and amorphous, it is difficult to differentiate it from a thrombus. The fibroblastic proliferation which extends from the base into the necrotic zone also contributes to the growth of the vegetation.

Since a wide area of normal vessel wall separated the vegetations, each may be considered to have developed independently. The small vegetations presented the appearance of early lesions. The destruction of the vessel wall proceeded concentrically from the intima outward. The maximal involvement was found in the most superficial portion of the intima. No changes were observed in the vasa vasorum. Therefore, it appears that the vegetations had their origin in the vascular endothelium.

These observations on the development of the vegetations are supported by the recent experimental studies conducted by Mac Neal and his associates.[9-11] In the early stages of *Str. viridans* infection of the rabbit, they found a widespread involvement of the endothelial cells. The infection tended to progress on the auricular surfaces of the mitral and tricuspid valves and on the ventricular surface of the aortic cusps. Mac Neal and associates stress that the diffuse invasion of the endothelial cells by the streptococci was by no means limited to the heart but could be found in the endothelial cells lining vascular channels elsewhere. If these changes also occur in man, an initial, potential lesion may be assumed to occur frequently in the pulmonary artery during the earliest stages of *Str. viridans* infection. If so, the initial lesions must usually heal with complete restitution.

Mechanical factors like the impact of blood, friction of approximating endothelial surfaces, contact with a larger amount of bacteria-laden blood, and pre-existing pathological changes are all known to be factors in the establishment of the vegetations in subacute bacterial endocarditis. There is also a great preponderance of left-sided cardiac involvement in this disease as illustrated by Libman's [12] finding of only one case with a lesion on the right side of the heart in a series of more than 100 cases of subacute endocarditis. On the other hand, the lesions caused by the more aggressive organisms in acute endocarditis occur on the right side of the heart more commonly. Libman found involvement of the right side of the heart in 26.8 per cent of his cases of acute endocarditis. These bacteria localize more often in hearts which have apparently not been involved previously, and they show some tendency to establish lesions in locations where the conditions of increased mechanical stress do not exist.[13]

In the present case, the lesions developed in the lesser circulation, involved an apparently healthy vessel wall, and occurred in an atypical location where undue mechanical stress could not be postulated. The *Str. viridans* localized, therefore, in a manner resembling that of the aggressive organisms encountered in acute endocarditis. Perhaps this *Str. viridans* possessed unusual virulence. This assumption also seems supported by the fairly rapid course of the disease which led to death 8 weeks after the first symptoms were noticed.

Str. viridans rarely produces an acute endocarditis (Held and Goldbloom [14]). Although one may be tempted to place the present case in this category, there are both clinical and pathological differences to be noticed between my case and those designated as acute endocarditis. This is illustrated by a comparative study of acute and subacute *Str. viridans*

endocarditis conducted by Held and Goldbloom.[14] They found the acute cases to be characterized clinically by a readily demonstrable acute infection which preceded the endocarditis. There was also a lack of anemia and a paucity of embolic phenomena. Pathologically, the lesions on the valves were predominantly ulcerative. In the present case, there was no history of preceding acute infection. The portal of entry of the infection was obscure, as it commonly is in subacute bacterial endocarditis. The anemia was profound. Due to the localization of the large vegetation in the beginning of the right main branch of the pulmonary artery, emboli could reach only the right lung and the latter was studded with infarcts. The slower progression of lesions due to *Str. viridans* was manifested by the marked fibroblastic reaction surrounding the infarcts and by the paucity of leukocytes. The arterial lesion was characterized by the formation of vegetations. Ulceration was apparent only after the vegetation had been removed. The marked proliferation of fibroblasts and the presence of occasional giant cells at the base of the vegetation also conformed to the type of lesion encountered in subacute bacterial endocarditis. The septic type of temperature in this case occurred at a time when pulmonary infarcts were present. It is not unusual to observe fever of this type in advanced cases of subacute bacterial endocarditis which are complicated by emboli.

Summary

A case of *Streptococcus viridans* endarteritis of the stem of the pulmonary artery is reported. There were no changes found in the heart and great vessels to account for the atypical localization of the vegetations.

The vegetations in the pulmonary artery consisted, to a large extent, of necrotic tissue arising from the vessel wall. This is in accordance with the observation that the vegetations in endocarditis are derived largely from tissue of the valves. Observations in this case suggest the inception of the vegetative lesions from the endothelium.

The atypical localization of the vegetations resembles that of the lesions encountered with the more aggressive organisms of acute endocarditis. This suggests an unusual virulence of the *Str. viridans* in this case. However, the appearance of the lesion and other important clinical and pathological findings are those usually found with subacute bacterial endocarditis.

The rarity of atypical pathological processes of the kind reported here emphasizes the importance of mechanical stress and of predisposing lesions for the localization of *Str. viridans*.

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DESCRIPTION OF PLATES

PLATE 87

FIG. 1. A large vegetation is shown to arise on the right side of the pulmonary artery at the level of its division and extends a short distance into the right main branch. Several small vegetations are present on the left side of the pulmonary artery and in its left main branch as indicated by the arrow.

FIG. 2. An inflammatory reaction is present at the base of a small vegetation. It is most intense adjacent to the bacterial mass and decreases toward the periphery. Hematoxylin and eosin stain. × 100.

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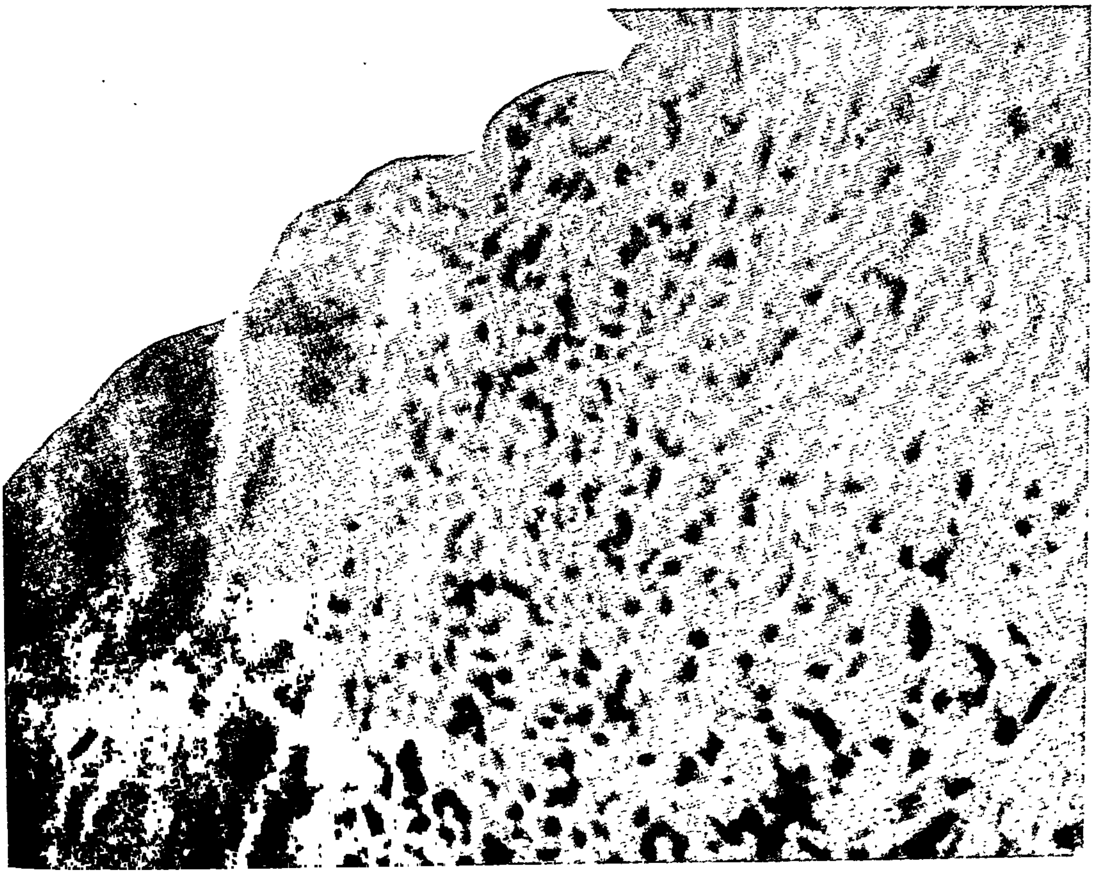

Rhoden Subacute Pulmonary Endarteritis

Plate 88

Fig. 3. Giant cells are present among the fibroblasts at the base of the large vegetation, a finding often encountered with the lesions of subacute bacterial endocarditis. Hematoxylin and eosin stain. × 450.

Fig. 4. The destruction of the elastic fibers at the base of a small vegetation is shown. Faintly staining remnants of elastic tissue within the vegetation suggest that the latter consists largely of tissue derived from the wall of the vessel. Weigert's elastic tissue stain. × 100.

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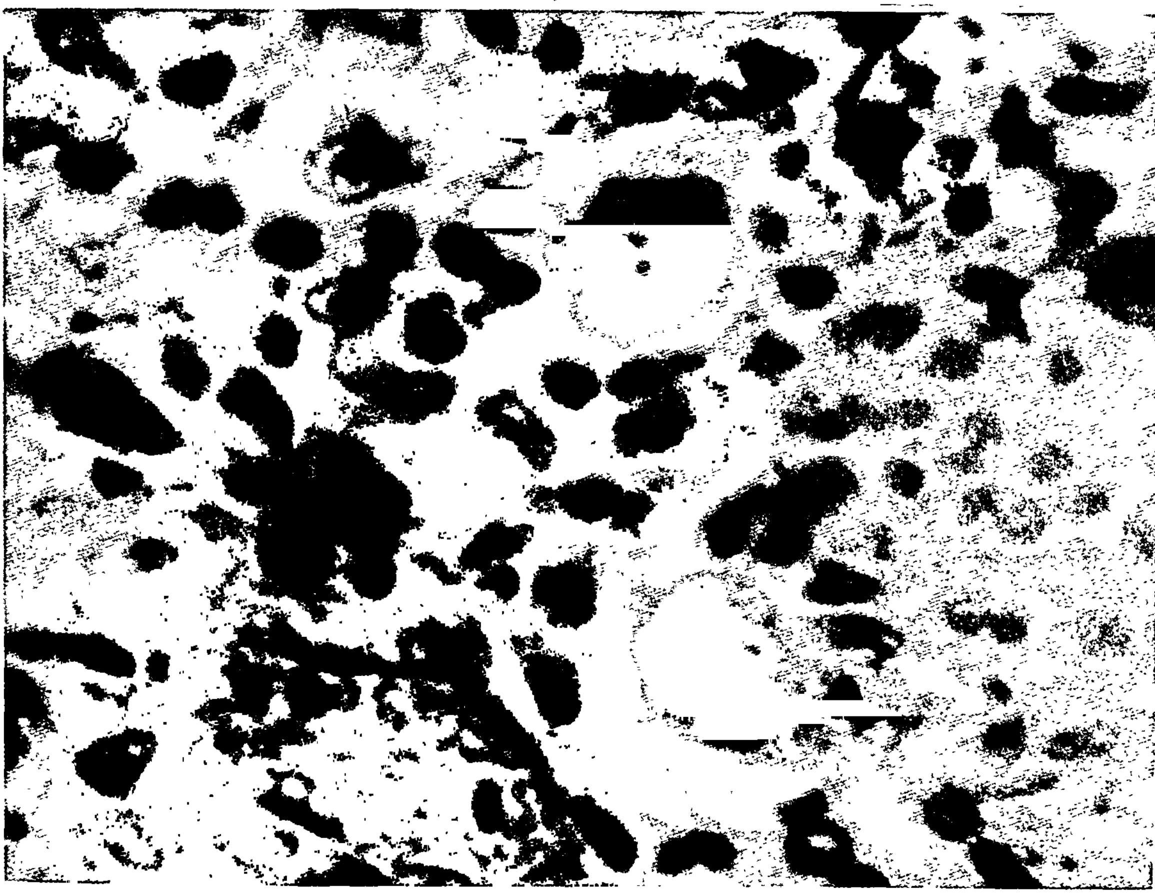

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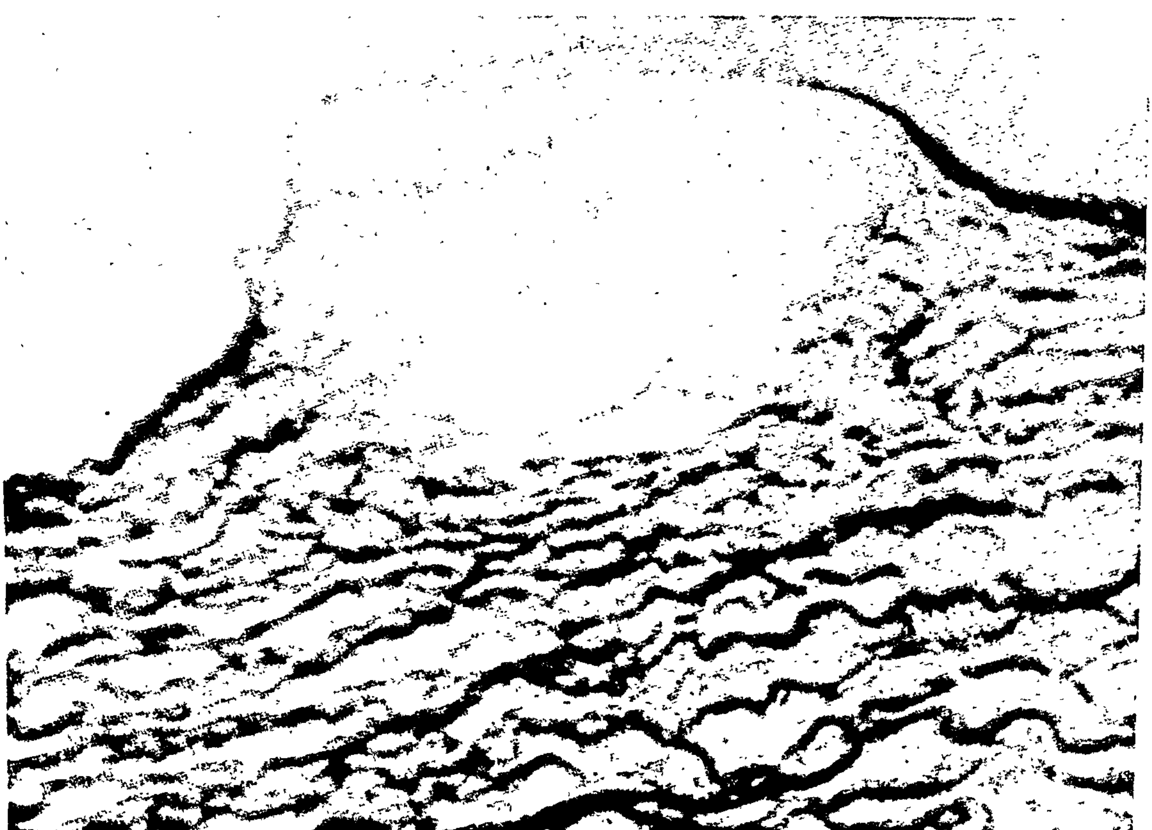

Rhoden Subacute Pulmonary Endarteritis

ACUTE DIFFUSE DEMYELINATING ENCEPHALOPATHY

Report of Two Cases *

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Considerable interest in diffuse demyelinating disease of the brain has arisen in recent years, not only in relation to its own etiology but also because of possible light which its study may cast upon its more chronic companion disease, multiple sclerosis, and to facilitate its clinical diagnosis. Following the original descriptions of the disease under a variety of clinical syndromes (encephalitis periaxialis diffusa, aplasia axialis extracorticalis congenita, symmetrical cerebral central lobar sclerosis, encephalopathia scleroticans, etc.[1]), new cases were reported in these sundry small categories, with many fine shades of distinction often becoming necessary in this system of classification.

An excellent descriptive study was contributed in 1924 by Collier and Greenfield.[2] Next came the statement in 1928 by Globus and Strauss [3] that at least a large group of these diseases could be considered as purely degenerative and therefore should be segregated under the heading of progressive degenerative subcortical encephalopathy. Their criterion for purely degenerative cases was absence of any cellular inflammatory reaction other than mild perivascular infiltration and gitter cell formation incidental to the clearing away of débris from dying cells. When Gasul [4] surveyed the literature in 1930 he found 72 cases (the number has risen rapidly since then). He concluded from that study that not all cases were purely degenerative, and that the etiology probably involved multiple factors.

From the clinical standpoint, meanwhile, Bouman [5] concluded, from a complete study of all available cases in the literature up to 1934, that there was no single group of cases with sufficient similarities among themselves and differences from other cases to be segregated as a distinct subdivision of diffuse sclerosis.

Finally, Ferraro [1] presented a rather exhaustive historical, clinical, pathological, and experimental study to show that all primary (and even secondary) demyelinating diseases of the central nervous system were essentially the same. He included multiple sclerosis, encephalitides following acute exanthemata, human and experimental deficiency conditions, and diseases following injection of such experimental toxins as potassium cyanide, tetanus toxin, and normal brain emulsion. As the situation stands today, most writers seem willing to accept the unifica-

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tion of at least the acute diffuse demyelinating diseases, if not of all demyelinating diseases. Papers are still being presented under one or another of the old syndromic titles, however.

Basis for Classification

The need for unification, at least for diffuse cerebral sclerosis, becomes apparent if one considers that:

1. "Specific" symptoms and neurologic signs may be produced by any of the diseases and depend only upon the rapidity, completeness, and anatomic location of the pathologic process. As indicated by Ferraro,[1] this point is fairly well accepted.

2. No constant difference in the above factors, and their resulting symptoms, can be found in the familial cases, in cases of any particular age group, or in any other subdivision of diffuse sclerosis. Disseminated sclerosis, of course, occurs in a decidedly older age group and with a more extensive and notoriously patchy distribution throughout the nervous system.

3. The pathologic findings are the same in all demyelinating diseases, as shown by Ferraro,[1] and the amount of terminal gliosis versus cyst formation and liquefaction, of axon cylinder preservation versus complete destruction, etc., depends upon the rapidity and severity of the process in the individual case rather than upon membership in one or another clinical syndrome.

4. Acute forms of cerebral multiple sclerosis are clinically and pathologically often indistinguishable from encephalitis periaxialis diffusa, as pointed out by Ferraro,[1] Meyer and Pilkington,[6] Reeves and Anderson,[7] and others.

5. "*Acute diffuse* sclerosis of *childhood*" may occur at *any age*,[1, 8, 9] may have *patches* anywhere in the central nervous system and involve gray matter as well as white,[1, 7] and may last as long as *15* years.[10] Thus it is difficult to delimit sharply so-called Schilder's disease from multiple sclerosis, when clinical as well as pathologic differences may disappear.

Etiology

It is significant that, as already implied, the demyelinating diseases with known etiology may be indistinguishable pathologically from those comprising the problem groups. Thus Ferraro [1] includes the demyelination of pernicious anemia, of bacterial toxins, of inorganic poisons, and of avitaminosis, in his system of classification, side by side with the syndromes of unknown etiology. Therefore it would seem unduly speculative for one to say that encephalitis periaxialis diffusa is inflammatory, or is degenerative, or even that there is but a single etiologic

factor. Indeed, it is more likely, by inference from the above facts, that the demyelinating diseases have a variety of causes and are thrown together because of a similarity in pathologic changes. When the various etiologic factors become known we shall have a satisfactory basis for classification; such a basis is of value in regard to prevention, treatment, and prognosis, all of which are of ultimately greater importance than fine distinctions in diagnosis.

A perusal of possible etiologic factors in individual cases of diffuse sclerosis does not clarify the situation. The experimental agents already mentioned (potassium cyanide, tetanus toxin, brain emulsion) have nothing in common with each other nor with canine distemper, human post-exanthematous encephalitis, vitamin deficiencies, nor with others listed below. Mackay [10] reported two cases following bacillary dysentery and pneumonia; Jervis and Kindwall [9] reported a case in an adult following severe chronic ergotamine poisoning. Winkelman and Moore [8] listed birth trauma, avitaminosis, acute infections, tuberculosis, adrenal atrophy, and carbon monoxide among causes reported or suggested in the literature. Several authors report a mild upper respiratory infection preceding the acute onset of symptoms, as in case 2 presented here. Diphtheria toxoid, administered to this patient, was followed by a convulsion within 2 hours, and it is difficult to conceive of its being the cause. In case 1 of this paper there was possibly an ascending infection, with chronic organizing ependymitis and fibrosis of the choroid plexus following surgical treatment of a lumbosacral myelomeningocele; the possibilities are discussed below. The last case of Winkelman and Moore [11] has a possible relation to a prolonged, difficult delivery requiring subsequent oxygen-carbon dioxide resuscitation.

Report of Cases

The following cases are presented as acute diffuse demyelinating encephalopathy of childhood, with certain unusual aspects.

Case 1

F. J. was a white female infant, delivered at term, with healthy parents and a normal, healthy brother of 5 years. There was no family history of nervous or mental diseases, and no congenital anomalies in the past four generations. This child was born with a lumbosacral myelomeningocele of 6 to 7 cm. in diameter and 2.5 cm. in height; a 2.5 cm. suboccipital meningocele; and widely separated suture lines and large fontanelles. The lumbosacral meningocele was tense, loculated, and translucent. Roentgenologic examination showed absence of the laminae and spinous processes from L.3 downward, including the sacrum. There was a defect of the skull which could allow a suboccipital meningocele. The skull appeared to show large loculi, which were interpreted as bony defects, a "Lückenschädel."

Laboratory findings were normal; Wassermann and Kahn tests were negative.

One month after birth the lumbosacral meningocele appeared larger and more

tense, and the fontanelles and sutures slightly more tense. The lower extremities were held in flexion and caused pain if extended, and there was complete skin anesthesia to pin-prick from L.2 downward. The levator ani and rectal and bladder sphincters were paralyzed.

Thirty-seven days after birth the lumbar meningocele was explored surgically and strengthened with lumbar fascia, under ether anesthesia; the dural sac was opened during the procedure, and nerve roots were seen floating in the fluid. Following operation there was progressive enlargement of the skull and there ensued mild fever ranging up to 99.5° F., which rose suddenly to 104° F. 1 week before death and continued to alternate between 103° and 106° F. until death. The child died at the age of 5 months, having reached a maximum weight of 13 lbs., 12 oz., with skull circumferences of 51.9 cm. suboccipitobregmatic, and 53.4 cm. occipitomental.

Clinical Diagnosis. Hydrocephalus.

Gross Findings at Autopsy

The body was that of a poorly nourished, under-developed infant showing tremendous enlargement of the skull, with broadly separated suture lines and large fontanelles. The lumbosacral scar showed no leakage. The various organs were essentially normal except for the central nervous system.

The brain weighed 1200 gm. It showed evidence of cerebellar herniation into the foramen magnum, and a distorted enlargement which had resulted from the differentially more rapid enlargement of the calvarium than of the base of the skull. The frontal lobes were therefore quite close to the brain stem, and the midcerebrum formed a great hump. The convolutions were broad, soft, often fluctuant, and so thin that on ordinary palpation the finger dropped through the surface into loculated cystic spaces containing clear, watery fluid. Sagittal sectioning revealed a swollen corpus callosum filled with cystic spaces containing clear fluid. Frontal sections showed a diffuse softening of the white matter throughout the hemispheres, with the tissue so edematous that its consistency was that of a very soft jelly. Isolated and loculated cysts of all sizes had formed. The process generally stopped abruptly at the cortex, although it sometimes also involved the inner cortical layers. Changes were most severe in the occipital region. The central gray masses were relatively free of this change. The ventricles were rather small and grouped close to the center of the brain, and all except the fourth ventricle were filled with semisolid, gruel-like, cheesy, white débris and had a firm zone of gliosis just outside the ependyma. A fibrosed mass of choroid plexus was seen in the roof of the fourth ventricle.

Hydromyelia was found in the upper half of the spinal cord, the central canal being irregularly dilated to 2 to 3 mm. Numerous tiny, fine, stringy adhesions connected the pia mater of the cord to the arachnoid, but the plane of cleavage was easily followed.

Histologic Findings

Throughout the white matter of the cerebral hemispheres and corpus callosum there was marked glial rarefaction, with only a few oligodendroglial and microglial cells and varying numbers of astrocytes present. These astrocytes were quite numerous in certain areas, notably near the basal ganglia, near some sections of cortex, and bordering cystic spaces. They tended to assume the gemästete form with a plump body of acidophilic cytoplasm and an eccentric nucleus. Clasmatodendrosis and other degenerative changes were seen only very infrequently, and then only in the most rarified areas. The cysts were found scattered throughout the white matter of both hemispheres and had replaced nearly all of the white matter in the left occipital lobe. Here they had interlacing trabeculae and walls of moderately dense glial tissue with numerous astrocytes. Several blood vessels here showed perivascular collars of lymphocytes and a few plasma cells; perivascular infiltration was insignificant elsewhere. An occasional scavenger cell contained droplets of neutral fat.

The cortex was relatively well preserved, except for some vacuolization and disarrangement of architecture in the occipital portion; and the basal ganglia, pons, and medulla showed no abnormality in sample sections. Demyelinization was confined to the white matter of the cerebral hemispheres and was almost complete, sparing only the arcuate fibers and an occasional islet. The axis cylinders, on the other hand, were destroyed only in the trabeculae and gliotic walls of cysts and near the ventricles.

Around the lateral and third ventricles there was a condensation of glia with a mixture of spongioblasts, astrocytes, and ependymoblasts with nuclei oriented more or less perpendicularly to the lumen. Islets and alveoli of ependymal cells were found only in the glial tissue around the third ventricle. Medial to the zone of gliosis, in all three ventricles, there was a lining of mesodermal granulation tissue, which in turn was lined by a mixture of compound granular cells, lymphocytes, and plasma cells. In the lumen there was a mass of fibrin and cellular detritus.

The choroid plexus of the third and fourth ventricles was partially buried in a mixture of glia and collagenous connective tissue, and the ependyma of the fourth ventricle was surrounded by a zone of gliosis and interrupted by numerous small areas of exuberant glial tissue protruding into the ventricle.

Around the pons and medulla there was fibrous thickening of meninges, and scattered groups of lymphocytes were present; elsewhere the meninges were normal.

In the spinal cord an intact layer of ependymal cells lined the dilated central canal, which had periodic (not segmental) outpocketings where the spinal cord became quite thin-walled. Some of the subependymal glia was arranged perpendicularly to the canal, but there was no increase in astrocytes. No parenchymal abnormality was seen. The pia mater was somewhat thickened and fibrous and had torn adhesions upon its surface.

Case 2

J. A. S. was a white male child, 1 year old, who had been well until the age of 9½ months, with normal birth and developmental history. He had had a mild upper respiratory infection for 1 week, was given a first injection of diphtheria toxoid, and had a severe generalized convulsion that same morning. A few hours later the temperature rose to 105° F., subsiding a few days afterward, and the patient then became comatose and remained so until death. He was first seen after the convulsion, when he was semicomatose, and was apparently blind and possibly deaf and displayed generalized spasticity with hyperactive reflexes.

The spinal fluid contained 7 lymphocytes per cmm., 64 mg. per cent protein, 741 mg. per cent sodium chloride, and 58 mg. per cent glucose. Blood and urine were normal. "Virus studies" were negative (type of study not stated).

Clinical Diagnosis. Encephalitis.

Gross Findings at Autopsy *

The head showed prominent frontal bossae and had the following measurements: suboccipitobregmatic circumference, 46.5 cm.; occipitofrontal, 45 cm.; biparietal diameter, 13 cm.; and bitemporal diameter, 10.5 cm.

The brain had edematous, flattened convolutions, was soft, and had an irregularly thin cortex which was accidentally pierced in a few places and immediaely poured forth clear, yellow fluid. After formalin fixation, frontal sections were made which showed a soft, spongy white matter with an extensive net of cavitation. The cortex was paper-thin in some areas. The central gray masses were somewhat soft but were without cavitation, and the cerebellum, midbrain, pons, and medulla appeared normal. There was moderate dilatation of the lateral and third ventricles and of the aqueduct of Sylvius. The other organs were essentially normal.

Histologic Findings

In the brain the basic change was a diffuse demyelinization and degeneration of the cerebral hemispheres, with involvement of the entire cerebral cortex and white matter, much of the basal ganglia on the left side, and a small area in the midbrain. In the left cerebral hemisphere the process appeared to have reached a termination with the formation of multiple cysts separated by glial trabeculae, while in the right hemi-

* Performed by Dr. William Brock.

sphere and the affected portions of gray matter in both sides the principal phase was that of glial proliferation.

In detail, the process appeared to start with an insidious diffuse increase of oligodendroglial and microglial cells (seen best in the right basal ganglia and in the pons), soon followed by disintegration of myelin sheaths and formation of numerous compound granular cells which took up the fat. This was quite marked in the right cerebral hemisphere. Edema separated the glial fibers, nerve cells and glial cells simultaneously degenerated, and perivascular infiltration of lymphocytes, plasma cells, a few neutrophils, and sometimes numerous compound granular cells occurred. When the degenerative process was not too rapid, there was an active proliferation of astrocytes, most of which were of the plump gemästete type, having a rounded, eosinophilic body, an eccentric nucleus, and either protoplasmic or fibrillary processes. Simultaneously with their proliferation, the astrocytes began to show degenerative changes such as clasmatodendrosis, extreme eccentricity or atrophy of the nucleus, and finally the production of a pink, oval body from the original cell. The process of degeneration may stop in the phase of gliosis and leave much of the tissue in that state, or it may pass through this stage or, if extremely rapid, never show gliosis at all but proceed directly to a final state of multiple cysts which are separated by thin trabeculae of glia and blood vessels.

Degenerating nerve cells sometimes became extensively ferruginated.

Comment

The first case is unusual in that the patient had developmental anomalies in the central nervous system; *i.e.*, a lumbosacral myelomeningocele, a suboccipital meningocele, and hydromyelia. Such patients usually develop hydrocephalus, but in this case the ventricles remained small. The diffuseness of the process in the brain, its limitation to subcortical tissue, the sparing of arcuate fibers, the relative severity of the occipital lesions, and the formation of cysts are all typical characteristics of acute diffuse subcortical demyelinating encephalopathy, or "encephalitis periaxialis diffusa."

In the second case the unusual feature is the nearly complete destruction of the cerebral cortex by continuation of the process. Cases of this type are well known and have been described by Alpers,[12] and others. This case presents the aspects of typical "sporadic, diffuse, cortical and subcortical demyelinating encephalopathy of infancy," according to Ferraro's nomenclature,[1] or "encephalitis periaxialis diffusa." The improbability of any direct relation of the disease to the administration of diphtheria toxoid has already been discussed.

SUMMARY

1. Two cases of acute demyelinating encephalopathy of infancy, with some unusual aspects, are presented.

2. In view of the failure of either clinical or pathologic evidence to delimit completely and sharply any single group of cases of demyelinating encephalomyelopathy from all other such cases, it is believed advisable to unify all such diseases and then subdivide the group according to etiologic factors when such factors become known. Apparently "natural," clinical or pathologic, subdivisions, such as multiple sclerosis, should be retained only when such classifying aids in prognosis and in statistical search for etiologic factors, and should not be maintained as a list of discrete entities from which the clinician must "make a choice" in diagnosing the individual case.

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REGRESSION PRODUCED IN THE MURPHY LYMPHOSARCOMA BY THE INJECTION OF HETEROLOGOUS ANTIBODIES *

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Such a marked impression was made upon me by the destruction produced in normal mammalian tissues by the injection of heterologous anti-tissue sera that a way was sought to apply this technic to neoplastic tissues. When this was assayed three aspects of the problem immediately presented themselves: first, how to secure a sufficient quantity of antigen and prepare it in a relatively chemically pure state; second, the production of a potent antibody; and third, the determination of whether or not this antibody would have sufficient differential destructive action on the lymphosarcoma without harm to normal tissues or lethal effect upon the host. These problems were, in reality, those which had faced workers in the field of tumor immunity from its first inception. So much so, in fact, that although experimental work had advanced to the stage where it seemed likely that the antigen-antibody reaction was sufficiently specific to be utilized against neoplasia, so many intricacies were found that heterologous antibody studies were largely abandoned. There were those who frankly believed they had produced effective anti-tumor antibodies; others objected not only to the methods but also to the results. In time the work on tumor immunity has come to be presented largely by workers who have successfully prevented the growth of tumors in animals by injections of homologous tissues prior to tumor implantation. This is hardly a practical end and one which allows of little further experimental extension.

The first of these difficulties seemed approachable when Murphy ‡ and Sturm [1] produced a rapidly growing lymphosarcoma-leukemia in rats, a tumor from which, in a short time, with the use of a number of animals, as much as a kilogram of neoplastic tissue could be readily obtained. With a sufficient supply of antigen on hand it was then possible to do chemical fractionation upon the tumor, as well as to test it for antibody production in a number of foreign species. Further, dosage in these species could be determined; and, lastly, the rate of tumor growth was sufficiently constant, when these animals were inbred, to make it usable as one against which the antibodies might be tested. Supplementary studies were done in order to test for the presence of antibodies in the sera by *in vitro* methods.

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‡ Dr. James B. Murphy of the Rockefeller Institute for Medical Research kindly furnished the original tumor from which transplants were carried in Wistar (Carworth strain) rats.

Materials and Methods

Tumor Antigen Preparation

The Murphy lymphosarcoma was chosen for this work because of its rapid growth, high percentage of takes, and lack of necrosis. Those tumors which grew well and showed no gross necrosis were used in antigen preparation. They were chosen on the eighth to twelfth day after transplantation. After rapid dissection under aseptic conditions and within 5 minutes of the death of the animal the tumor was placed in thin slices in a sterile Petri dish set in solid carbon dioxide. The tumors thus harvested were then placed at —20° C. and held until 500 to 1,500 gm. were secured, ordinarily for 1 week. All antigens to be described were derived from this material.

The first tests were done with a whole tumor antigen prepared by grinding the frozen tumor. It was then extracted in cold physiological saline solution for 24 to 48 hours, centrifuged, and the supernatant liquid decanted. The supernatant liquid was used as one fraction. The residue was then washed several times with cold saline solution by centrifugation and the resulting solid portion resuspended in cold saline and used as a second fraction. These two antigens were injected intramuscularly into rabbits over a period of approximately 2 months at intervals of 5 to 6 days. The amounts injected ranged from 2 to 15 ml. in the case of the soluble antigen, and 1 to 8 ml. in the case of the insoluble antigen. On the tenth day following the final injection these rabbits were bled from the heart and the serum thus obtained was used.

Later chemical fractionation of the tumor was undertaken by two different methods and throughout the remainder of the experiments these fractions, together with whole minced tumor, were used as antigens. When the chemical fractions were given, the amounts were measured in milligrams. When whole minced tumor was used, the number of milliliters used was calculated to correspond to the number of milligrams, on the basis of the presence of 8 per cent solid protein in the tumor. Intravenous injections were found to be unsatisfactory and intramuscular injections were then resorted to exclusively. In relatively few instances these were found to cause sterile abscesses.

Tumor Antigen Injections

The chemical methods employed were derived chiefly from attempts to obtain different protein fractions. Thus one procedure was to prepare antigens by primary precipitation of the protein from a phosphate buffer extract of the frozen ground tumor, by acidification to pH 4.2 with acetic acid (method I). The abundant precipitate so obtained was taken off by centrifugation in the cold. The filtrate or supernatant

liquid was neutralized by ⅓ stepwise saturations with ammonium sulfate. A second procedure (method II) was straight stepwise saturation of the original material with ammonium sulfate at pH 7.0. These two methods with a classification of their precipitates are shown in Table I. Table II gives the nitrogen-phosphorus partition of the various frac-

TABLE I

The Two Methods of Chemical Treatment of Fresh-Frozen Lymphosarcoma (Murphy) Used to Obtain Antigens

Method I			Method II		
Fraction number	Method of preparation	Possible fraction	Fraction number	Method of preparation	Possible fraction
1. L. S. HAc	Ppt. from phosphate extract by acidif. to pH 4.2 with acetic acid	Nucleoprotein, any denatured protein	1. L. S. ⅓ sat., $(NH_4)_2SO_4$	Ppt. from ⅓ sat. of phosphate buffer extract	Nucleoprotein (?), pseudo- & euglobulins
2. L. S. ⅓ sat., $(NH_4)_2SO_4$	Ppt. from ⅓ sat. of neutralized filtrate from No. 1	Euglobulins, pseudoglobulins	2. L. S. ⅔ sat.	Ppt. by ⅔ sat. filtrate from No. 1	Globulins
3. L. S. ⅔ sat.	Ppt. from ⅔ sat. of filtrate from No. 2	Globulins	3. L. S. sat.	Ppt. by sat. filtrate from No. 2	Albumins, including contaminating serum albumins, and hemoglobin
4. L. S. sat.	Ppt. from sat. filtrate from No. 3	Albumins, including contaminating serum albumins, and hemoglobin			

TABLE II

Nitrogen-Phosphorus Partition of the Various Fractions Obtained in Methods I and II

Fraction number	Murphy Lymphosarcoma (rat)		
	N mg. %	P mg. %	P:N
HAc *I* from saline, no acetone treatment	13.9	1.5	1:9
HAc *I* from saline, treatment acetone, ether	14.6	1.44	1:10
Remainder of fractions not treated with acetone, ether			
Method I.			
1. HAc *I* from phosphate	11.0	1.78	1:6
2. ⅓ saturated	14.0	0.44	1:32
3. ⅔ saturated	16.0	0.099	1:161
4. Saturated	13.6	0.32	1:42
Method II.			
1. ⅓ saturated	9.44	1.50	1:6
2. ⅔ saturated	15.8	0.32	1:49
3. Saturated	13.6	0.50	1:27
Nucleic acid fraction from ⅓ saturated	11–12.6	6.70	1:1.64 1:1.88

tions. All precipitates were finally dried in the lyovac apparatus and injected into animals after resuspension in physiological saline.

Antigen Dosage

In order to ascertain the most effective dose an experiment was conducted in which the amounts of antigen given per injection were 1.0, 10.0, 50.0, and 100.0 mg. These were injected into rabbits at 3 and 4 days intervals for ten injections. Ten days following the last injection the rabbits were bled and the antibody titer determined by precipitin tests. Those rabbits receiving 1.0 and 10.0 mg. dosages were usually found to have a low titer of 1:256 or less, while in those receiving 50.0 and 100.0 mg. the titers tended to be higher, in the range of 1:1024. The day following this first bleeding, injections were begun again. Five of these were given, all of the rabbits receiving large doses, starting with 50 mg. and increasing to 250 mg. Ten days following this final injection they were bled again. In all instances they showed titers of 1:512 to 1:1024.

On the basis of the above experiment it was concluded that the large dosages were the more effective, and subsequent series of injections were conducted on that basis. A typical plan for antibody production follows:

Day of Injection	Amount of Injection
1st	50 mg.
3rd	100 mg.
6th	100 mg.
9th	100 mg.
13th	200 mg.
17th	200 mg.
22nd	200 mg.
27th	200 mg.
32nd	250 mg.
37th	250 mg.
47th to 50th	Bled

In addition to the rabbits employed, one series of ducks and two series of guinea-pigs were used. Equally satisfactory titers were obtained with the ducks, but since the rabbits were just as effective and far easier to care for, the ducks were not used. The guinea-pigs were found to be unsatisfactory, primarily because only low titers in the range of 1:64 could be obtained. Furthermore, it was impossible to obtain as large quantities of serum in return for comparable amounts of antigen administered.

The serum was obtained from the rabbits under aseptic conditions and after preliminary clotting was centrifuged from the clot as quickly as possible. It was then stored in sterile ampules until ready for use.

The sera were tested for precipitins only after each lot was tested on the animal which bore a tumor. The precipitin tests were set up by preparing the antigens according to their type.

Method of in Vitro Tests

For whole tumor antigen, fresh tumor was obtained and minced. A small portion of the minced tumor was then shaken thoroughly with cold normal saline and allowed to stand for at least 1 hour at 4° C.

The cloudy supernatant fluid was then withdrawn with a pipette and considered as undiluted antigen. The chemical fractions presented another problem inasmuch as they were largely insoluble. They were prepared ordinarily as 1 per cent solutions in normal saline, this concentration being taken as undiluted antigen. In some instances an attempt was made to put the more insoluble fractions into solution by adjusting the pH with 0.02 N. NaOH. In others they were prepared in 0.1 M. phosphate buffer solution rather than normal saline.

Since it is known that heat slowly inactivates precipitins, none of the sera were heated in these tests. The varying dilutions of antigen in 0.1 ml. amounts were layered onto 0.1 ml. amounts of serum in small precipitin tubes. In all instances suitable controls consisting of either saline or phosphate buffer solution, as determined by the preparation of the antigen, and of normal serum were included. The tubes were placed in a water bath at 37° C. for 1 hour. Sometimes they were examined for the presence of interfacial rings at the end of this period. This reading was never so high as that obtained after placing them in the ice box overnight. The preliminary reading was therefore discontinued and a single reading made following a minimum of 18 hours at a temperature of 4° C.

Method of in Vivo Tests

There was a certain percentage of spontaneous regressions with this lymphosarcoma (about 10 per cent, after considerable inbreeding of animals for tumor take and using just-weaned to 4 weeks post-weaned animals). For this reason care was exercised in the method of antibody testing. The plan was to test litter mates in equal numbers, half with control serum, half with antibody serum. The animals were inoculated from a fresh, viable tumor and the tumors given 4 to 5 days to establish themselves. After it was certain that the tumor was growing—they measured 12 to 18 mm. in longest diameter at this time—the animals were picked indiscriminately and numbered. The tumors were measured and after the animals were returned to their cage they were withdrawn at random and the first half then received the antiserum, and the second half, control serum. One observer made daily measurements without knowledge of the animals' numbers. A second identified the animals and recorded the measurements.

A preliminary series of six tumor-bearing rats received antiserum from fraction 1 of method I directly over the site of the tumor. All of these tumors regressed with heavy fibrosis about them. Six tumors, injected with control serum, grew without restraint. Since it could not be ascertained whether the fibrosis attendant upon the antibody injection was nonspecific, all subsequent injections were placed in a sub-

cutaneous region away from the tumor. The antiserum was administered in amounts ranging from 1 to 5 ml. at a single injection, and over a period of 10 days as much as 5 to 14 ml. was given.

There was amazingly little reaction from the antibodies derived from any of the antigens. In a large series of animals injected with rabbit serum containing antibody two died from what appeared to be sensitivity to the serum. Another reaction occurred—induration of the subcutaneous tissue at the site of antiserum inoculation. This was most common from fractions 3 and 4 antisera prepared by either method, and following massive doses into the subcutaneous region.

Results

The results are presented in three sections: (1) effects observed in tumor-bearing animals; (2) gross and microscopic observations on the treated animals; (3) observations in connection with *in vitro* precipitin tests.

Effects on Tumor-Bearing Animals

The percentage of regressions in the first series of experiments is shown in Table III. There was a 10 per cent regression in 83 animals which were not treated or which received normal rabbit serum. Seventy-

TABLE III

Summary of 10 Experiments Showing Results of Antibody Treatment of Rat Lymphosarcoma-Leukemia (Murphy); All Animals, Both Controls and Those Treated, Are Included

Test group	Serum titer and quantity	Total tumors	Number which grew at usual rate	Number whose growth rate was retarded	Number which regressed	Average interval before death in retarded group	Regression
Rats which received no serum	None	46	41	None	5	—	10%
Rats which received normal rabbit serum	None 7–14 ml.	37	33	None	4	—	10%
High titer antibody serum from antigen fraction 1	256–1024 8–14 ml.	22	3	7	12	19	54%
Low titer antibody serum from antigen fraction 1	64–128 5–10 ml.	28	7	17	4	25	14%
Antibody serum from antigens of all other tumor fractions	64–1024 7–14 ml.	25	16	4	5	40	20%

Regressions in all control animals	10%	83 animals
Regressions in all treated animals	28%	75 animals
Regressions in high-titer, high-dose animals	54%	22 animals

five animals treated with antibodies obtained in all fractions, and both minimal (5 ml.) and maximal (14 ml.) quantities of serum, gave 28 per cent complete regressions. Of these, the high-titer, high-dose animals, 22 in number, gave 54 per cent regressions.

It was discovered after completing the data in Table III that it was possible to correlate the percentage of regressions with the height of titer and dosage. If either the dosage or titer is low the tumor will not regress.

Fraction 1 antiserum made the most effective antibody.

TABLE IV

A Later Series of Experiments Than Those Shown in Table III

Antigen fractions	Antibody titer and quantity	Total tumors	Number which grew unchecked	Number retarded	Number which regressed	Average interval before death in retarded group	Regression
Normal rabbit serum (controls)	8–14 ml.	41	38	None	3	—	6%
Fraction 1. HAc	256–1024 8–14 ml.	21	2	7	12	19	57%
Fraction 1. HAc	64–128 5–10 ml.	23	11	5	7	28	30%
All other fractions	64–1024	25	16	4	5	40	20%

A later group of experiments, which gave almost identical results, is shown in Table IV. A somewhat higher average of total regressions, using all titers and dosages of antiserum, was obtained in this series.

The results, when the serum was used against animals previously inoculated with the tumor intraperitoneally (leukemic phase), are shown in Table V.

The growth curves (longest tumor diameter), of 15 nonregressing tumors treated with normal rabbit serum, are shown in Text-Figure 1. Text-Figure 2 shows an equal number of litter mate animals which bore tumors treated with anti-tumor serum. The difference in pattern is striking. Some animals which were treated with ineffective dosage and with sera of low antibody titer are included.

Gross and Microscopic Observations on Animals Treated with Anti-Lymphosarcoma Antibodies

Figure 1 shows a section taken from an animal which had received 12 ml. of normal rabbit serum. Those tumors, which were subsequently to regress under antibody treatment, showed softening within 24 hours following the first injection. The control tumors remained firm, pink, and extended in size. The regressing tumors became pale, and upon

palpation there was definite softening of the center of the tumor—evident necrosis. At 5 days to 1 week there was definite induration about the tumor, and if there was to be complete regression, there was a steady decrease in size. As may be observed from Text-Figure 2, all of the tumors did not regress. Even though it was not always possible to produce regression, often a great retardation in the rate of tumor progression would be brought about. On the average, death occurred later in all groups of treated animals with subcutaneous implants than in the controls. This was very notable in some groups, being particularly striking in those treated with antisera from all fractions other than 1.

TABLE V

Intraperitoneal Inoculations: Results of Antibody Treatment of Animals Inoculated Intraperitoneally with Rat Lymphosarcoma-Leukemia (Murphy)

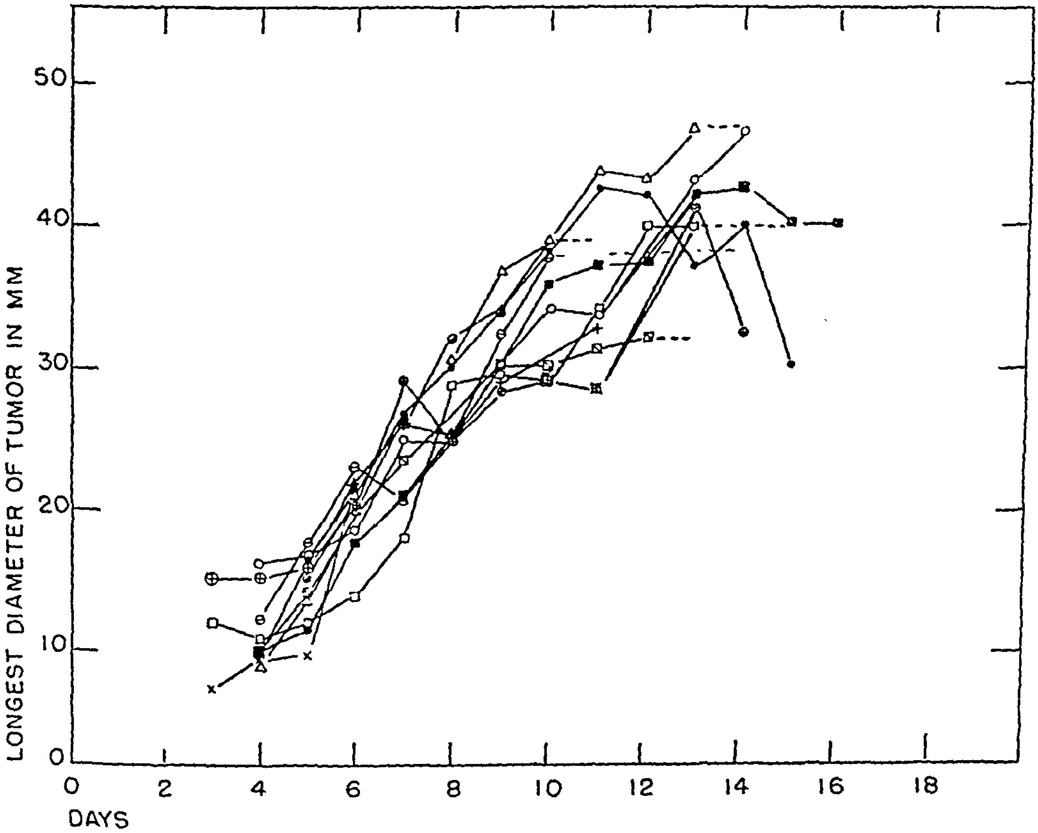
Test group	Serum titer and quantity	Total animals	Recovered	Retardation of clinical course	Number which died	Average interval before death
						days
Rats which received no serum	None	11	None	None	11	7
Rats which received normal rabbit serum	No titer 6–12 ml.	9	None	None	9	8
Antibody serum from antigen 1	Low and high 7–15 ml.	8	38%	—	5	8.5
Antibody serum from other fractions	Low and high 7–15 ml.	6	17%	1–25 days	5	9

In this group the average interval before death was 40 days, whereas the controls had died in an average of 13 days. One of these treated animals lived beyond the 50th day and very slowly developed an enormous tumor, which measured 70 by 55 by 20 mm.

Tumors which had been treated locally with the earlier and less effective antiserum showed viable lymphosarcoma cells heavily surrounded by a matrix of fibrous connective tissue (Figs. 2 and 3). This is quite similar to the picture seen late in other treated tumors, which, while they had not regressed, were greatly slowed in growth. These, also, showed scar tissue about them and a reduction in mitotic figures. Tumors which were successfully caused to regress with antiserum showed massive necrosis and fibrosis at the tumor site (Figs. 4, 5, and 6) with few to moderate numbers of giant cells. These two patterns were compared to those of a number of tumors which had spontaneously regressed (Figs. 7 and 8). There was no outstanding difference. The tumors which had spontaneously regressed tended to have less fibrous

connective tissue, more giant cells, and somewhat larger areas of calcification in the necrotic areas.

Examination of other tissues, with special attention to lymphoid tissue and bone marrow, showed no consistent differences in the organs of control, normal serum-treated, or antiserum-treated animals, except that almost all animals which had received maximal dosage of antibodies showed some hyperplasia of the spleen and bone marrow. There



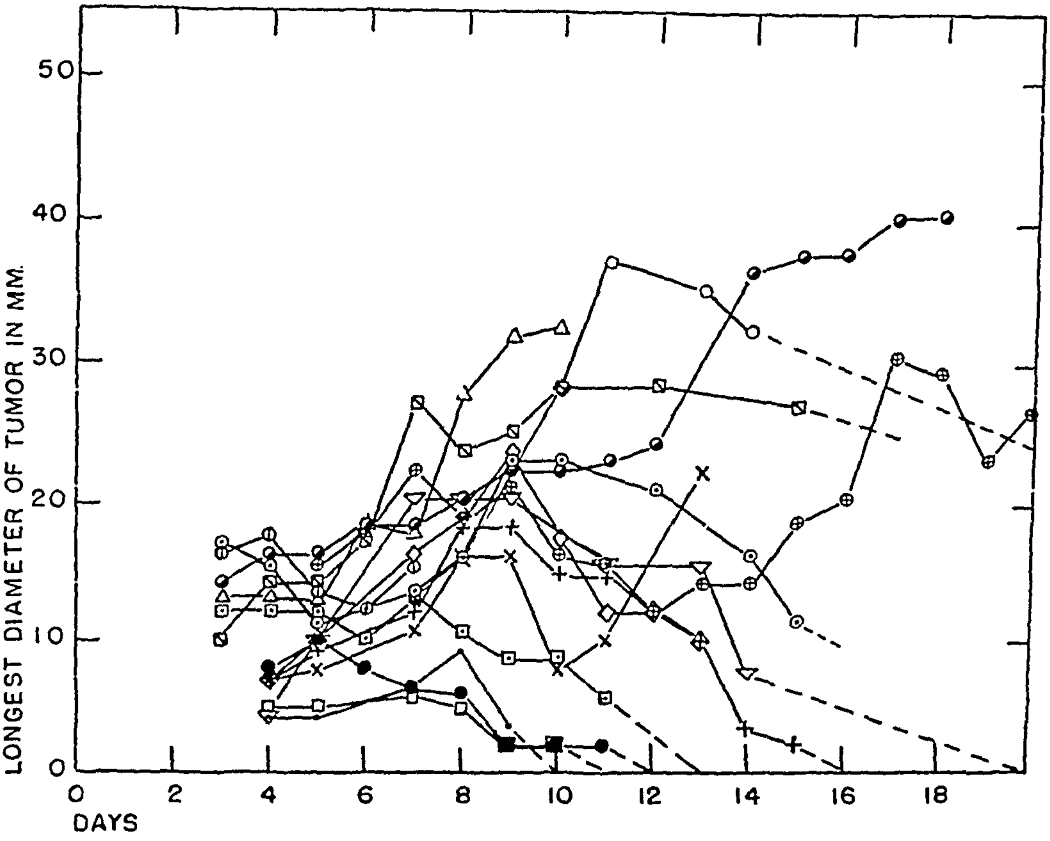
Text-Fig. 1. Curves made from the daily longest measurements of 15 tumors treated with normal serum.

were no late effects from this, since the tissues did not evidence necrosis or fibrosis. In areas of skin and subcutaneous tissue which had been removed from the indurated areas previously mentioned, there was considerable edema, mild inflammatory reactions, and some overgrowth of fibrous connective tissue.

Antigen-Antibody Reactions in Vitro

It is not possible to detail all of the results from the *in vitro* tests with the antigen-antibody reactions. Small 50 by 6 mm. tubes were used and dilutions of antigen from 1:2 through 1:1024 were obtained by doubling. After chilling in the ice box the tests were read in reflected

light; the precipitin was distinguished from the solid particles which tended to confuse the results. It was found that rabbits gave the highest consistent titers and the height of the titer depended upon a number of factors. Of these, the most important observable were: (1) the dosage, (2) length of inoculation period, and (3) the animal itself. Thus the highest titers were obtained in animals which received enorm-



Text-Fig. 2. Curves made in similar manner to those in Text-Figure 1 from 15 tumors treated with antisera.

ous doses of antigen over a long period, and of these animals some were high antibody producers, others were not.

Discussion

The present work encourages one to inquire further into the possibility of anti-tumor sera. There were deliberately sought, in this work, experimental conditions as nearly ideal as possible: abundant antigen, direct protein precipitation methods, massive doses of antigen over long periods in the heterologous species. In likelihood, the greater portion of antigen protein was derived from the lymphosarcoma nuclei, since they constituted the greater portion of the tumor mass. The antibodies against these proteins proved most potent in destroying the tumors. It is curious that the antibodies against constituents theo-

retically derived from the cytoplasm were most useful in prolonging the life of the animal when the tumor was not immediately destroyed.

It was discovered very early that here, as in bacterial diseases, potent antibody of sufficient quantity must be injected early to be of any value whatsoever. The injection of antibody serum in the late stages of the disease did not produce a single instance of regression. This suggests that, even in the face of some satisfying results, the antibodies prepared were not of great potency. Or if so, it must take large quantities to destroy effectively large numbers of individual tumor cells.

It was observed in those animals which bore spontaneously regressing tumors that the mechanism whereby lymphosarcoma cell destruction and subsequent fibrosis was produced came about with relentless certainty. The mechanism of this regression, if discoverable, should be of great value.

While it seemed possible to produce regression in transplanted lymphosarcoma with heterologous antiserum, it is not certain whether a similar result might be obtained in spontaneous tumors. These may differ markedly in their behavior from transplanted tumors. A statistically significant result was obtained in this work and it is hoped that the results soon will be tested against spontaneous tumors in animals.

Summary and Conclusions

1. By the use of rabbit anti-lymphosarcoma serum, it was possible to produce both retardation in rate of tumor growth and a significant reduction in mortality in rats bearing the Murphy lymphosarcoma.

2. Grossly, those tumors which regressed showed rapidly progressive necrosis, followed by fibrosis at the tumor site.

3. Microscopic examination of the tumor sites showed destruction of lymphosarcoma cells with massive fibrosis. This reaction did not differ markedly from that which occurred in spontaneously regressing tumors.

4. It was found necessary to utilize serum of high titer and in considerable quantity to effect those results.

5. The chemical fraction found most useful as an antigen was determined to be that which contains the nucleoproteins.

6. A prolonged immunization period, in rabbits, was needed to produce serum of high-antibody titer.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

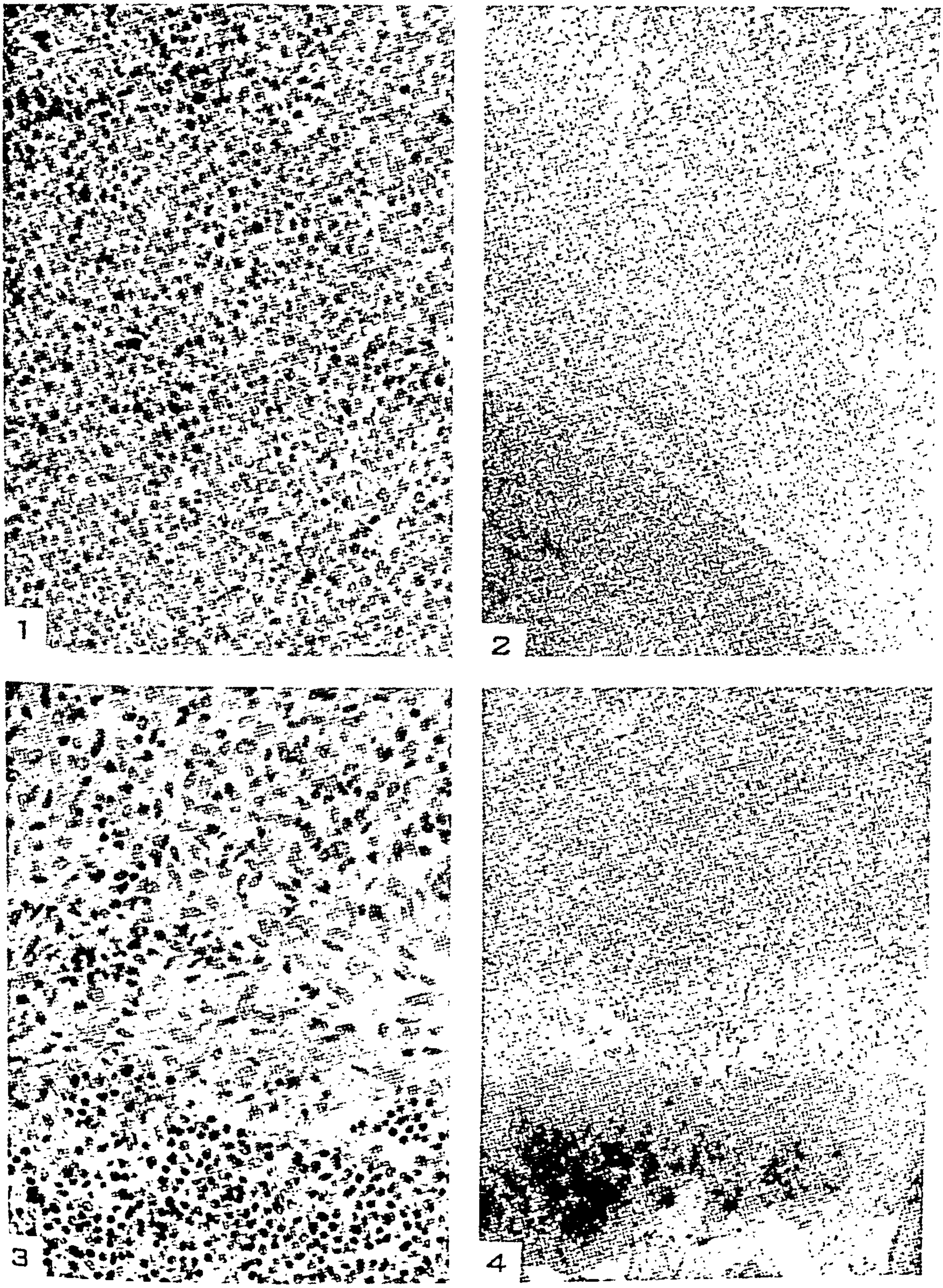
PLATE 89

FIG. 1. Section removed on the eleventh day from a tumor in an animal which had received 12 ml. of normal (control) rabbit serum. The cell pattern is indistinguishable from that of tumors taken from animals which received no serum. × 250.

FIG. 2. This section of lymphosarcoma was removed from an animal which had received antiserum injections at the tumor site. Although there is abundant peri-tumor fibrosis, there is little necrosis of tumor cells. × 70.

FIG. 3. Higher-power field from the same tumor as shown in Figure 2. The lymphosarcoma cells are below. ×250.

FIG. 4. Section from tumor site in an animal which had received 14 ml. of high-titer antiserum (fraction 1, method I) subcutaneously and not at the tumor site. × 70.



Nettleship Regression in Lymphosarcoma

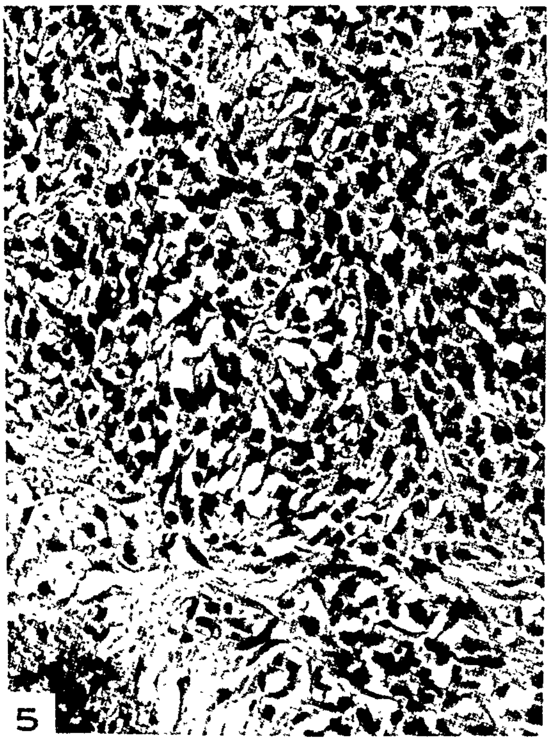
PLATE 90

FIG. 5. Taken from same section as Figure 4. Only fibroblasts and an area of necrosis (in the lower left corner) are seen. × 250.

FIG. 6. Another tumor site from a high-titer, antiserum treated animal, taken on the tenth day. × 250.

FIG. 7. Low-power field of a section of tumor which had spontaneously regressed, taken on the 13th day. There is fibrosis and necrosis. The granular black areas are areas of calcification. × 70.

FIG. 8. From the same section as Figure 7, showing giant cells in the lower portion of the field. × 250.



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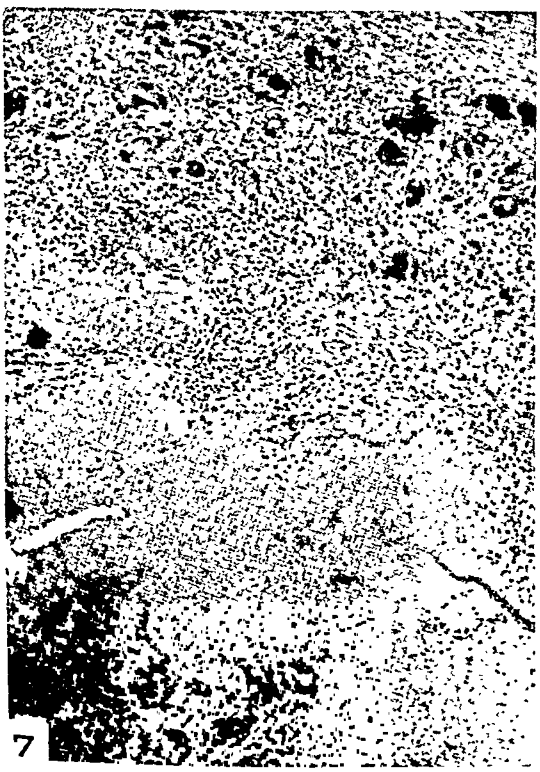

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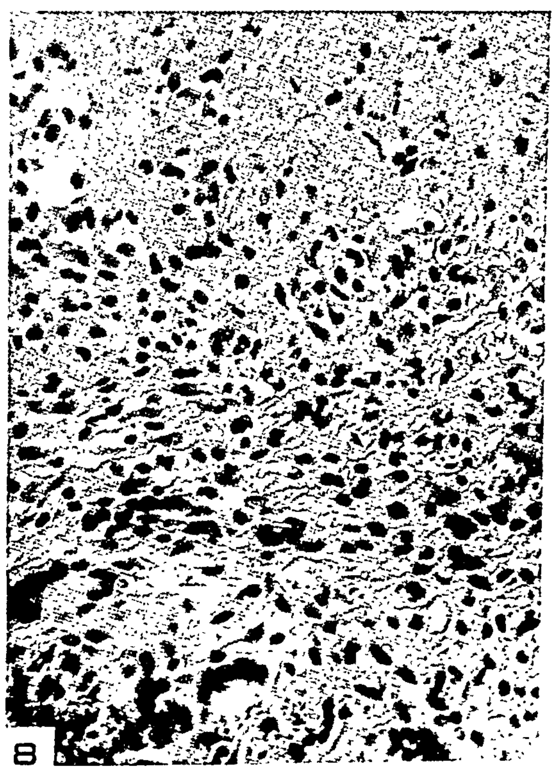

8

Nettleship Regression in Lymphosarcoma

THE DIAGNOSIS OF GRANULOMA VENEREUM FROM FROZEN SECTIONS STAINED WITH POLYCHROME METHYLENE BLUE *

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Since Donovan's original observation [1] of the constant presence of specific intracellular inclusions in the lesions of granuloma venereum, the use of tissue smears stained by variations of the Romanowsky method has been a standard laboratory procedure in the diagnosis of this disease. But the indifferent staining of these bodies with hematoxylin and eosin, their failure to retain the Gram stain, and the variable results obtained by modifications of the Romanowsky stain long discouraged the use of histopathologic methods for the study of this disease. Practical and reliable technics for the demonstration of these organisms in sections were not reported until 1937, when Pund and Greenblatt, using Delafield's hematoxylin [2] and eosin, and Dieterle's silver impregnation method,[3] were able to describe the specific character of the intracellular parasitism of the disease.[4, 5]

To augment the simple, rapid, smear technic, and the section methods of Pund and Greenblatt as laboratory procedures in the study of granuloma venereum, one further observation is recorded here. In frozen tissue sections, stained with Terry's polychrome methylene blue, the histologic features of the disease are brilliantly demonstrated.

Terry's neutralized polychrome methylene blue [6, 7] is made up as follows:
1. Stock solutions, in neutral distilled water:

A.	12% anhydrous potassium carbonate, C.P.	100 cc.
B.	1% methylene blue (Grübler's or Merck's medicinal) .	1000 cc.
C.	10% acetic acid, by volume	100 cc.

2. Titration. Dilute 1 cc. of solution C with 15 cc. of distilled water. Add as an indicator 5 drops of phenolphthalein. Heat to boiling and determine how much of solution A is required to neutralize. Mark this quantity on bottle A.
3. Alkalinization. Into a 100 cc. graduate, place that quantity of A which is equivalent to 1 cc. of C, add enough of B to make 100 cc. and mix thoroughly.
4. "Polychroming." Of the alkalinized methylene blue, 25 cc. are placed in each of four 1 oz. bottles. These are stood unstoppered in cold water. This is brought to a boil in about 10 minutes and kept boiling during the procedure. Remove the bottles, one by one, 15, 20, 25, and 30 minutes after boiling has begun.
5. Neutralization. To each 25 cc. of the polychromed stain add 0.25 cc. of solution C.

Filtration is usually unnecessary and should not be carried out for 1 or 2 days. The four different bottles contain stain of different polychromatic values, the richest one being that which was allowed to digest longest. Any of the four will be satisfactory. Generally speaking, the richer the stain in colors, the better the

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results with unfixed tissues. Tissues either fixed rapidly in heated formalin, or fixed routinely in formalin, stain readily with the solutions poorer in color. The neutralized stains are stable.

For the type of tissue usually obtained from lesions clinically resembling granuloma venereum, formalin fixation, either rapid or routine, is recommended, although unfixed tissues may be used if desired. Frozen sections, cut at 20 to 25 μ, are floated or dipped in the dye for a few seconds, the time required for staining being determined by trial. The sections are then washed and mounted on a slide in water. From time to time a drop of water may be added to the edge of the coverslip to keep the preparations wet. Tissues stained by this method cannot be satisfactorily preserved, but they may be kept long enough to allow careful study.

A detailed description of the frozen section technic is to be found in the article by Forbus.[7]

The appearance of the intracellular parasites in these preparations is essentially similar to that obtained with other methods, but certain differences are apparent. The flatness of contour and the cellular disruption seen in smears are eliminated, and the shrinkage and possible distortions resulting from dehydration, clearing and mounting in the other section methods are avoided. Large parasitized mononuclear cells, 20 μ or more in diameter, are readily recognized under the high-dry objective, and they can be given detailed study under oil immersion. The clear cystic cytoplasm of the invaded cells often makes them conspicuous even in relatively thick preparations (Fig. 1). A peripheral distribution of bodies within the intracytoplasmic cysts is sometimes observed (Fig. 2) but, perhaps because of the wide range of focus possible, this feature does not have the prominence observed in paraffin sections. In such cells a slight alteration of the focal plane will obliterate the "ringed" pattern of the bodies (Fig. 3). Some intracytoplasmic cysts are completely filled with the organisms (Figs. 4 and 5). Less heavily infected cells may contain no cysts. Although parasitism is essentially a feature of mononuclear cells, occasionally single bodies can be seen in polymorphonuclear cells (Fig. 5). Extracellular bodies can be recognized singly and in small groups.

The Donovan bodies are stained a delicate blue. The staining is diffuse, with no strong tendency toward polar staining, and no metachromatic granule or nucleus can be demonstrated within the body. The peripheral zone, or capsule, is clear and unstained (Figs. 4 to 7). The bodies have a variable coccobacillary shape, and occasionally diplococcal forms are observed within a continuous capsular membrane (Fig. 7), indicating a process of fission. The organisms usually

measure 0.6 to 1.8 μ in length, and 0.6 to 0.8 μ in diameter, with the investing capsular zone being about 0.3 μ in thickness. Extremely elongated bacillary forms have not yet been seen with this technic.

The granules of mast cells are stained a reddish purple in these preparations, and cannot be confused with the organisms. The Donovan bodies appear, however, so similar to other bacterial forms that the diagnosis of the disease cannot be made on the basis of their morphology and staining characteristics alone. The characteristic parasitism of mononuclear cells must be observed. Although this feature of the disease is strikingly similar to that observed in leishmaniasis and histoplasmosis, the smaller size of the Donovan bodies enables them to be differentiated from the other agents on a morphological basis alone.

If granuloma venereum always presented a straightforward clinical picture, the smear technic and the slower section methods of Pund and Greenblatt [4] would be adequate procedures for the diagnosis and study of the disease. But the tendency of this condition to simulate other processes, especially in the cervix uteri where the lesions are frequently diagnosed as carcinoma,[5] makes it desirable to have available a rapid and efficient biopsy technic. This need is satisfied by the frozen section method. This compares in effectiveness with the standard procedures and, for laboratories serving outside hospitals and receiving fixed materials for study, it is the only rapid method available. It has the disadvantage that the preparations cannot be satisfactorily preserved, so that it has to be supplemented by the longer section methods in order to obtain permanent records. So little tissue need be used in the procedure that the usual specimen removed for biopsy will serve for both procedures. For photomicrographic study and camera lucida drawings, carefully prepared sections are superior to any other preparations.

Summary

The use of frozen sections stained by Terry's polychrome methylene blue is recommended as an aid in the diagnosis and study of granuloma venereum. The technic satisfies the need for a rapid and efficient method suitable for all types of tissues.

The details in the morphology of the Donovan bodies, especially the capsular zone, are demonstrated better by this technic than by any of the other section methods. These features are briefly described.

The thickness of the sections allows a wide range of focus, and it is often possible to obtain a three dimensional study of the parasitized mononuclear cells. This affords a particularly good opportunity for the study of the peculiar intracytoplasmic distribution of the bodies. A

comparison is made of the pattern observed in frozen sections with that seen in sections prepared by other methods.

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DESCRIPTION OF PLATE

Plate 91

The photomicrographs, using Wratten filters B and K1, are of frozen sections stained with Terry's neutralized polychrome methylene blue.

Fig. 1. Parasitized cell. The cystic cytoplasm stands out against the dark background of the thick preparation. × 1275.

Fig. 2. Parasitized cell, showing peripheral distribution of Donovan bodies within intracytoplasmic cysts. × 1235.

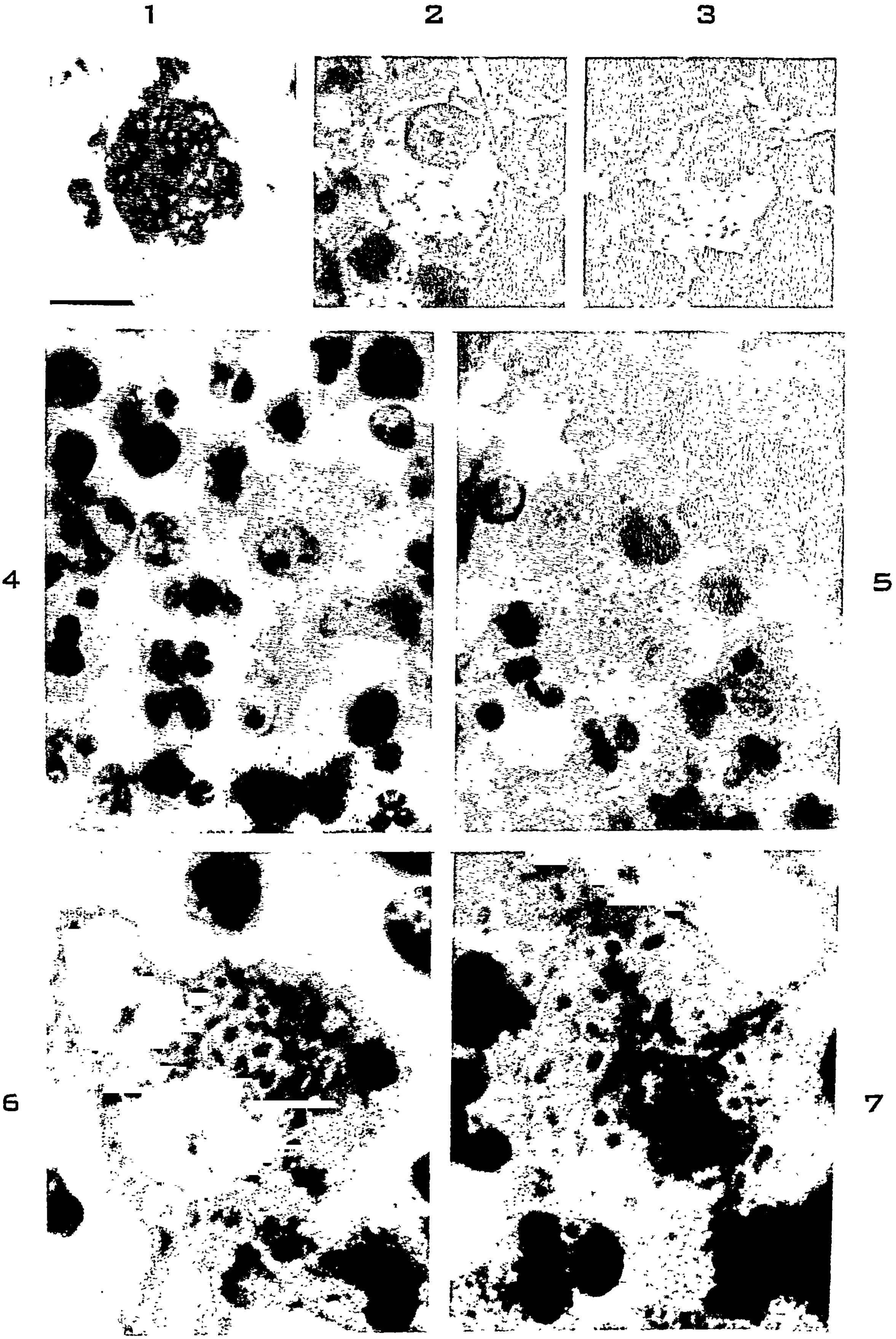
Fig. 3. The same cell, at a slightly altered focus. The ringed pattern of the bodies has disappeared. × 1235.

Fig. 4. The character of the inflammatory exudate and the mononuclear parasitism are shown. × 1275.

Fig. 5. Several parasitized mononuclear cells are included. Below the large central mononuclear cell a polymorphonuclear cell is seen containing a single body. × 1275.

Fig. 6. A portion of Figure 4 enlarged to show details of the morphology of the Donovan bodies. × 2550.

Fig. 7. A portion of Figure 5 enlarged to illustrate details in the structure of the Donovan bodies. The pleomorphism of the organisms, the capsular zone, and diplococcal forms are demonstrated. ×2550.



Margolis Diagnosis of Granuloma Venereum

ARTERIAL OCCLUSIONS PRODUCED BY EMBOLI FROM ERODED AORTIC ATHEROMATOUS PLAQUES *

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In a recent autopsy of a man with advanced arteriosclerosis, changes were observed in some of the small and medium-sized arteries of the kidney, spleen, pancreas, and thyroid. In the lumina of these vessels were spaces with the shape of cholesterol crystals and a few slit-like endothelium-lined channels. Foreign body giant cells partly surrounded many of the cholesterol crystal spaces. These occluding lesions were found in arteries having an external diameter of from 55 to 900 μ. Their appearance suggested that emboli, containing large cholesterol crystals, had lodged in these vessels and undergone organization. Since there was advanced erosion of the atheromatous plaques in the aorta of this man, it was believed that these eroded plaques were the source of the emboli.

It is known that the contents of an atheromatous plaque may serve as emboli if the surface of the plaque undergoes erosion,† [1, 2] and occlusion of coronary arteries by similar emboli has been described.[3]

Since there have been no recent studies on this subject, this investigation was made.

Material and Methods

Two hundred and sixty-seven autopsies were selected for review. Of these, 233 had been diagnosed as having "advanced arteriosclerosis" in the aorta. All of these had many atheromatous plaques in the intima of the aorta, and in some instances the atheromatous areas had eroded and were partially covered with mural thrombi. Thirty-four other cases were reviewed because the description of the aortas suggested that the degree of atherosclerosis was actually "advanced" although in the anatomic diagnoses it was called "moderate."

Of the 267 subjects, 191 were males ranging in age from 33 to 92 years, and 76 were females of from 35 to 86 years. One hundred and forty males and 57 females were over 60 years of age. The average age of all patients was 64.9 years. Sections of the kidney were ex-

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† The term "erosion" is used in this paper to describe the process of breaking down of the intimal surfaces of atheromatous plaques. This is seen commonly in the aortas, and occasionally in other arteries as well, of people with advanced arteriosclerosis. Since the flowing stream of blood is the force which removes the material of the plaque, the term "erosion" is appropriate in both geologic and pathologic senses. It is unfortunate that the term "ulceration" is used in some texts to describe this process, as the lesions produced are not inflammatory in origin and have no etiologic or histologic resemblance to ulcers.

amined in 244 cases, of the pancreas in 256 cases, of the spleen in 251 cases, of the adrenal in 109 cases, of the thyroid in 16 cases, and of the prostate in 20 cases. Often more than one section of an organ was examined. In selected cases serial sections were cut and the vascular changes studied in detail.

Vascular occlusions were found in 9 of these autopsies, yielding an incidence of 3.4 per cent among the 267 patients with advanced arteriosclerosis of the aorta.

Most of the sections were stained with hematoxylin and eosin; certain of them were stained also with Weigert's stain for elastic fibers. Frozen sections were cut of formalin-fixed tissues from case 1, and stained for fat with Sudan III.

The dimension of an artery was obtained by measuring the external diameter of the media with a calibrated ocular micrometer. This was done in the sections of fixed, paraffin-imbedded material.

The term cholesterol crystal spaces is used to describe the slit-like spaces which remain in tissues at the sites of cholesterol crystals dissolved during the preparation of the sections.

Report of Cases

Case 1 is given in detail. In Table I are listed the age, cause of death, blood pressure, and the degree of arteriosclerosis found at autopsy in each of the 9 cases. The distribution and frequency of the arterial occlusions are summarized in Table II.

Case 1

A white male, 61 years old, had a blood pressure of 180/100. He died with symptoms of coronary thrombosis.

At autopsy (autopsy no. 11016; by Dr. C. M. Flory) the entire aorta was markedly arteriosclerotic. In even the ascending portion and arch of the aorta some atheromatous plaques were superficially eroded and covered with mural thrombi. In the remainder of the aorta the intima was a mass of confluent atheroma, most of which was eroded, and covered with thrombi. Calcification was scanty. The coronary arteries contained many large atheromatous plaques and in places their lumina were stenosed. The heart weighed 710 gm.; its left ventricle was greatly enlarged. Healed infarcts were present in the myocardium. The iliac, splenic, celiac, right renal, and other medium-sized arteries were dilated and tortuous. The left kidney was a fluid-filled sac, and its ureter occluded by a scar. The right kidney weighed 155 gm.; its cortical surface was indented by deep wedge-shaped scars. In the area between the scars the cortex was of normal thickness. The small renal arteries were thick-walled. The spleen, pancreas, and thyroid gland appeared normal.

Histologic Observations

Great Vessels. In the intima of the aorta, 1 cm. above the aortic valve, were thick atheromatous plaques filled with cholesterol crystal spaces and lipid-filled macrophages. In the lower portions of the aorta the intima was 2.5 mm. thick and filled with structureless, gruel-like material containing many cholesterol crystal spaces. In some areas the intimal lining was eroded, and mural thrombi containing crystal-shaped spaces were attached to the atheromatous material (Fig. 1). About some of the cholesterol crystal spaces were foreign body giant cells.

Right Kidney. The cortex of the right kidney was slightly thinner than normal, and beneath the wedge-shaped depressed areas the glomeruli and tubules were atrophic or hyalinized. The large and small renal arteries were thick-walled. Their intimas were greatly thickened, and there was much reduplication of the internal elastic lamina. The arterioles were hyalinized.

TABLE I

Clinical and Pathologic Data on Nine Cases with Emboli from Atheromatous Plaques

Case number*	Autopsy number	Age	Cause of death	Blood pressure	Arterio-sclerosis of aorta	Erosion of atherosclerotic plaques in aorta			Mural thrombi in aorta
						Thoracic aorta	Abdominal aorta	Site not stated	
1	11016	61	Coronary thrombosis	180/100	+++	++	+++		+++
2	8526	70	Cellulitis of neck	184/94	+++	0	++		0
3	9146	58	Coronary thrombosis	?	+++			+++	++
4	9855	69	Pyelo-nephritis	240/120	+++			++	++
5	10204	48	Carcinoma of stomach	140/80	+++	0	+++		++
6	10453	72	Coronary thrombosis	170/120	+++			+++	?
7	10633	67	Broncho-pneumonia	175/100	+++	+++	+++		0
8	10644	69	Peritonitis after lapa-rotomy	130/70	+++			+++	+
9	11226	50	Coronary thrombosis	160/108	+++	+++	+++		++

* All patients were males.
0 = No lesion.
\+ = Slight changes present.
++ = Moderate changes present.
+++ = Advanced changes present.
? = Information not given.

Cholesterol crystal spaces, partly surrounded by giant cells, and a few slit-like vascular spaces were seen in the lumina of the arteries in each of the 5 blocks of tissue examined. In 174 arteries over 50 μ in external diameter, 20, or 11 per cent of the total, contained these lesions. The arteries involved varied in diameter from 58 to 880 μ.

In a typical lesion the intima of the artery was hyperplastic and the entire lumen was filled with loose connective tissue surrounding cholesterol crystal spaces and a few thin vascular channels. The crystal-shaped spaces were frequently bordered by foreign body giant cells.

TABLE II

Distribution and Frequency of Arterial Occlusions

Case number	Heart	Lung	Liver	Spleen	Pancreas	Kidney	Adrenal	Thyroid
1	0	0	0	++	+	++	0	+
2	0	0	0	+	+	+	—	0
3	0	0	0	+	+	+	—	—
4	0	0	0	0	+	0	0	—
5	0	0	0	0	0	+	—	—
6	0	0	0	+	+	0	—	0
7	0	0	0	0	+	0	—	—
8	0	0	0	+	0	0	—	—
9	0	0	0	+	+	0	—	0
Totals	0	0	0	6	7	4	0	1

0 = Organ contained no occluded arteries.
+ = Organ contained one or few occluded arteries.
++ = Over 10% of arteries in the organ were occluded.
— = Organ not examined histologically.

A large lesion is shown in Figure 2. This was in an artery measuring 880 μ in diameter. In its lumen were four large cholesterol crystal spaces, about two of which were foreign body giant cells. Several small vascular channels ran through the hyperplastic intima which surrounded the crystal spaces. Many hemosiderin-filled macrophages were present, suggesting either that this was an organizing embolus or that there had been hemorrhage into this lesion. On following this lesion in serial sections, the pattern shifted rapidly, but the component parts—vascular channels, cholesterol crystal spaces, and giant cells—remained. This occluded vessel lay in the base of a large wedge-shaped area of cortical atrophy.

Another occluded vessel is shown in Figure 3. This measured 825 μ in diameter and was filled with small vascular channels and by large

cholesterol crystal spaces partly surrounded by giant cells. A few of these crystal-shaped spaces contained a homogeneous pink-staining, protein-like material, apparently the matrix of the crystals.

An earlier lesion is shown in Figure 4. A large branch of an artery measuring 920 μ across was plugged by cholesterol crystal spaces. These were partly surrounded by giant cells and loosely attached to the wall by hyperplastic intima. The mass projected into the lumen of the smaller vessel from the larger one.

Another type of lesion, consisting largely of vascular channels, is shown in Figure 5, A to E. This occlusion began at the bifurcation of an artery with diameter of about 600 μ. The first portion of the lesion consisted of a V-shaped group of vascular channels surrounding a few cholesterol slits (Figure 5-A). The bifurcation of this vessel is seen in Figure 5-B and its two channels in 5-C. In Figure 5-D the branches are separated and the lower branch is filled with cholesterol crystal spaces. These vessels lay in the base of a wedge-shaped area of cortical atrophy (Fig. 5-E). Many small branches of these arteries were filled similarly with crystal-shaped spaces. No hemosiderin was seen.

Many smaller vessels in the kidneys were also involved. These arteries varied from about 60 to 200 μ in diameter. In some the occlusions were similar to those in larger arteries. In others (see Figs. 8 and 9 of small splenic arteries) the cholesterol crystal spaces lay in the innermost portion of the thickened intima, and the vascular channel passed to one side. In some sections macrophages with vacuolated cytoplasm lay near the crystal spaces, which were partly surrounded by giant cells.

Frozen sections were cut from formalin-fixed blocks of the kidney tissue, stained for fat with Sudan III and counterstained with hematoxylin. Most of the cholesterol crystals did not remain *in situ* even in the frozen sections. In a few vessels, however, thin, rectangular crystals were seen. Most of the lipid which stained with Sudan III was in the outer portions of the intimas of the occluded vessels. The cholesterol crystal spaces were always found in the former lumina of the vessels, and often were surrounded by lipid-free tissue. In other vessels some lipid was present about the cholesterol crystal spaces, but the amount was never so great as in the outer layers of the intima.

Left Kidney. The glomeruli and tubules of the left kidney were atrophic and fibrosed, and the cortex and medulla were very thin. The arteries had thick walls but contained no lesions.

Spleen. The splenic pulp and malpighian bodies were normal. In the small arteries the intima was hyperplastic, and reduplication of the internal elastic lamina was prominent. The arterioles were hyalinized.

Two blocks of the spleen were available for study. In single sections

of both blocks were 63 arteries with a diameter of 50 μ or over, of which 12, or 19 per cent, had these lesions in their lumina. The occluded vessels varied from 58 to 540 μ in diameter. Three occluded splenic arteries (the largest measuring 540 μ) are shown in Figure 6. In branches of these arteries also the lumina were filled with cholesterol crystal spaces.

Figure 7 is a small branch of a larger occluded artery; the foreign body giant cells about the crystal spaces are clearly shown. Lesions of different histologic appearance than that seen in Figure 7 are shown in Figures 8 and 9. The vessel illustrated in Figure 8 measured 84 μ in diameter. Its muscularis and adventitia were vacuolated and contained scattered lymphocytes. Cholesterol crystal spaces, partly surrounded by giant cells, lay against the intimal surface of one side of the artery. In the second vessel (Fig. 9) the crystal-shaped spaces were attached to the surface of the intima and were surrounded by giant cells. A large vascular channel remained.

Pancreas. Histologic examination of the pancreas showed the parenchymal cells and islets to be normal. Fibrous tissue was excessive between the lobules of the gland. Intimal hyperplasia was marked in the medium-sized and small arteries. The arterioles were hyalinized.

Four arteries, varying in size from 77 to 270 μ in diameter, contained these lesions. The lumen of the largest was filled with intimal tissue in which were several slit-like vascular channels and two cholesterol crystal spaces surrounded by giant cells. The lesions in the other arteries were similar. One segment of a small artery contained a typical lesion; the next segment of the vessel was almost obliterated by intimal hyperplasia and by an accumulation of macrophages with lipid-filled cytoplasm.

Thyroid. The small arteries of the thyroid gland were thick-walled and their intimas hyperplastic. In the lumina of two small arteries were cholesterol crystal spaces and giant cells.

Other Organs. No occluding vascular lesions were found in the heart, lungs, liver, or adrenals.

Relation of Arterial Occlusions to Arteriosclerosis and Other Diseases

The 267 cases can be divided into three groups. In 63 cases erosion of atherosclerotic plaques in the aorta was not noted. No arterial occlusions containing cholesterol crystals were found in these cases. The second group consisted of 147 cases in which the erosion was of slight or moderate degree. Only two instances of these arterial occlusions (cases 2 and 4) were found in the group, an incidence of 1.3 per cent. In the third group the erosion of the plaques in the aorta was marked. Such advanced erosion was found in 57 cases, of which 7, or 12.3 per

cent, had arterial occlusions containing cholesterol crystals. The absence of this lesion in patients without advanced arteriosclerosis of the aorta has been substantiated by studying our routine autopsies. No arterial occlusions containing cholesterol crystals have been found in over 200 of these patients.

The occlusions have not been found among the very old, but in patients from 48 to 72 years of age, with an average age of 62.5 years. All were males. Of the 9 patients with this lesion, 7 had hypertension, 8 had narrowing or occlusion of coronary arteries, and 5 had myocardial infarcts. No patient, however, had diabetes, and only 2 had positive serological tests for syphilis.

Origin and Development of the Arterial Occlusions

The only early lesion found was a thrombus containing large cholesterol crystal spaces. This was attached to the wall of a medium-sized artery in the kidney of case 5 (Fig. 12). It seemed almost certain that these crystals were dislodged from eroded atheromatous plaques in the aorta and carried as emboli into this vessel.

The mass of cholesterol crystal slits projecting into the lumen of a medium-sized vessel of case 1 (Fig. 4) was an older, better organized lesion. There was a moderate degree of intimal proliferation about these crystal-shaped spaces. No remnant of the thrombus remained. Although many other medium-sized arteries were occluded in case 1, no crystal-shaped spaces were found within recent thrombi. The presence of many hemosiderin-filled macrophages in one occluded vessel (Fig. 2) suggested that a thrombus might have been present and organized.

Fully developed, well organized lesions were found in the medium-sized arteries in cases 1, 2, 3, and 9. The lumina of these vessels were filled with cholesterol crystal spaces surrounded by a few foreign body giant cells and intimal tissue. This type of occlusion is shown in Figures 2, 3, 5, and 6.

In every case except case 5 some small arteries measuring from 50 to 200 μ in diameter (Figs. 7 to 11) were partially or completely occluded by these lesions. No arterioles were affected. The histologic appearance of these occlusions in small vessels was more variable than that in the medium-sized vessels. In some of the small arteries the lumina were plugged by masses of cholesterol crystal spaces partly surrounded by giant cells. Figures 7 and 10 illustrate such lesions. Many of the small occluded arteries in case 1 and other cases were actually branches of larger obstructed vessels.

Emboli of cholesterol crystals from eroded aortic atheromata seemed to explain the origin of all of these lesions satisfactorily. In some small arteries, however, the cholesterol crystals lay against the hyperplastic

intimas of vessels in which the vascular channel was not occluded (Figs. 8, 9, and 11). It seemed possible that such cholesterol crystals might have been formed from the lipids in the thickened intima of the vessels. They probably, however, were examples of advanced recanalization of a vessel previously occluded by cholesterol crystal emboli.

It is believed that the arterial occlusions containing cholesterol crystal spaces are the result of organization of emboli from eroded aortic atheromata. The mass of cholesterol crystals, mixed with lipid and thrombus material, is torn loose by the flow of blood and is carried into a medium-sized or small artery, where it lodges. About this embolus a thrombus forms and organizes. The blood clot is removed, but the cholesterol crystals remain and are encased by intimal tissue and foreign body giant cells. Recanalization of the thrombus takes place between or beside the crystals, forming slit-like vascular spaces. In a completely organized lesion the artery is occluded by cholesterol crystals, often surrounded by foreign body giant cells, slit-like vascular spaces, and hyperplastic intimal tissue.

The only anatomic changes associated with these arterial occlusions were found in the kidney where the renal parenchyma supplied by these obstructed vessels was atrophic, forming depressed, wedge-shaped cortical areas (Fig. 5-E). No changes attributable to these vascular lesions were observed in the pancreas, spleen, or thyroid.

Experimental Production of the Lesions

An unfixed human aorta was selected in which atherosclerosis was marked. Soft yellow material was scraped from several of the plaques and suspended in 5 cc. of physiologic saline solution. Microscopic examination of this fluid revealed many large, clear, thin rhomboidal crystals having the characteristic shape of cholesterol crystals. Fat droplets and red blood cells also were present.

Two and one-half cc. of this material was injected into the ear veins of 2 rabbits. One animal was killed after 24 hours. In its lungs many small arteries were occluded by masses of red blood cells, polymorphonuclear leukocytes, and large cholesterol crystals. The other animal was killed after 7 days. Many small pulmonary arteries were also occluded. In these vessels the cholesterol crystals were no longer surrounded by leukocytes but by foreign body giant cells and hyperplastic intimal tissue (Figure 13).

Discussion

The embolic theory of the origin of these arterial occlusions has been discussed. Another explanation is that the crystals formed *in situ* in the hyperplastic, lipid-rich intima of the arteriosclerotic vessels and

that the entire process is an unusual form of arteriosclerosis. If the interpretation of the histologic appearance of these lesions presented previously is correct, the arteriosclerotic hypothesis seems untenable.

Additional evidence against the theory of the formation of the crystals *in situ* in the arteries is the fact that in 63 patients with advanced arteriosclerosis of the aorta but with no erosion of aortic atheromatous plaques, crystal-containing arterial lesions were not found despite the fact that the splenic, pancreatic, and renal arteries were often very thick-walled and contained much lipid in their hyperplastic intimal tissues. In the 147 patients with slight or moderate erosion, the lesion was found in only 1.3 per cent. However, of the 57 cases with advanced erosion of atheromatous plaques in the aorta, 7, or 12.3 per cent, had these lesions. This suggests that embolism rather than arteriosclerosis is the cause. It may be argued, however, that the arteriosclerosis was actually more marked in the latter groups. This certainly was true in the aorta, but there was no histologic evidence that the arteriosclerosis in the smaller vessels was more severe in one group than in the others.

Another argument against the theory of formation of these crystals *in situ* is the location of the crystals in the arteries. If the lipid in the thick intimal tissue of arteriosclerotic arteries were to crystallize, one would expect to find vessels with crystals in their intimas as well as vessels in which the lumina were filled with crystals. Crystals have not been found in the intimas of medium-sized unoccluded arteries. In the similarly sized occluded vessels the crystals were always in the luminal portion of the vessel. This suggests that these large crystals entered the vessels as emboli.

In certain small arteries, however, crystals have been observed in the intimas of vessels in which large lumina were present. It would be dogmatic to say that these lesions were the result of partial recanalization of a vessel previously occluded by cholesterol crystal emboli, although this is a possible explanation. The thickened intimal tissues of these small arteries occasionally contained lipid-filled macrophages. In such vessels cholesterol might have crystallized from this lipid *in situ*.

The possible effects of these vascular occlusions should be emphasized. In case 1, where about 10 per cent of the renal arteries were occluded by these lesions, anatomic changes in the kidneys were produced. These consisted of many wedge-shaped areas of cortical atrophy. In the other cases where medium-sized renal arteries were occluded, similar wedge-shaped areas of cortical atrophy were also found distal to the occluded vessels. No infarcts related to these arterial occlusions have been observed.

It is possible, however, that cholesterol crystal emboli may produce

infarcts in the kidney or spleen, and that gangrene of a toe or some other portion of a lower extremity, occurring in an old person with advanced arteriosclerosis, may occasionally be caused by cholesterol crystal emboli.

Conclusions

Arterial occlusions produced by emboli from eroded aortic atheromatous plaques have been found in the small and medium-sized arteries of the spleen, pancreas, and kidney. In the nine cases in which such lesions were observed the frequency and the distribution of the lesions were variable. In one case the lesions were numerous, involving 19 per cent of the splenic and 11 per cent of the renal arteries; in other cases only a few vessels were involved.

In a typical lesion the lumen of the artery was filled with large cholesterol crystal spaces surrounded by hyperplastic intimal tissue and a few foreign body giant cells. In the kidney these occlusions caused wedge-shaped areas of cortical atrophy.

The intravenous injection of material containing cholesterol crystals, obtained from an atheromatous human aorta, has produced similar lesions in the arteries of the lungs of rabbits.

I wish to thank Mr. Julius Mesiar, who made the photographs which illustrate this paper, and Miss Helen Hirschbein for their technical assistance.

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DESCRIPTION OF PLATES

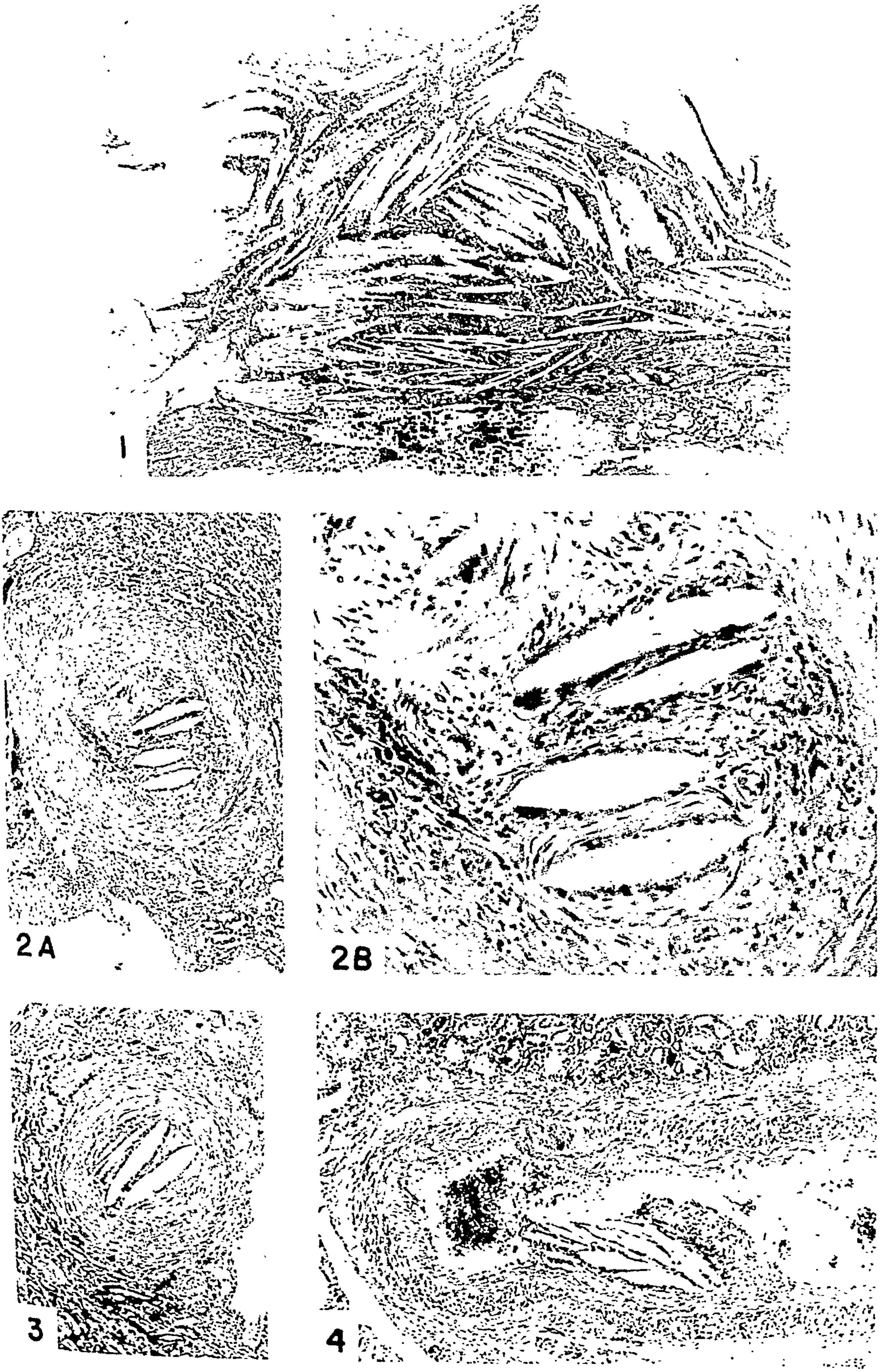
Plate 92

Fig. 1. Case 1. Surface of eroded atheromatous plaque in aorta. The mass of cholesterol crystal spaces is mixed with red blood cells; the surface of this lesion is very rough. × 50.

Figs. 2-A and 2-B. Case 1. Occlusion of a medium-sized renal artery. In *Figure 2-A* the entire artery is seen; this measured 880 μ in diameter. The large slit-like spaces in the former lumen of the vessel are cholesterol crystal spaces. × 50. In *Figure 2-B* giant cells can be seen at the ends of some of the crystal-shaped spaces. The dark masses of cells in the left side of the figure are hemosiderin-filled macrophages. × 160.

Fig. 3. Case 1. Occluded medium-sized renal artery. In this artery, which measured 825 μ in diameter, the large spaces are cholesterol crystal spaces, the small slits, vascular channels. × 50.

Fig. 4. Case 1. Intimal proliferation about a mass of cholesterol crystal slits in a medium-sized renal artery. In subsequent sections these slit-like spaces fill the lumen of a large branch of this vessel. × 50.

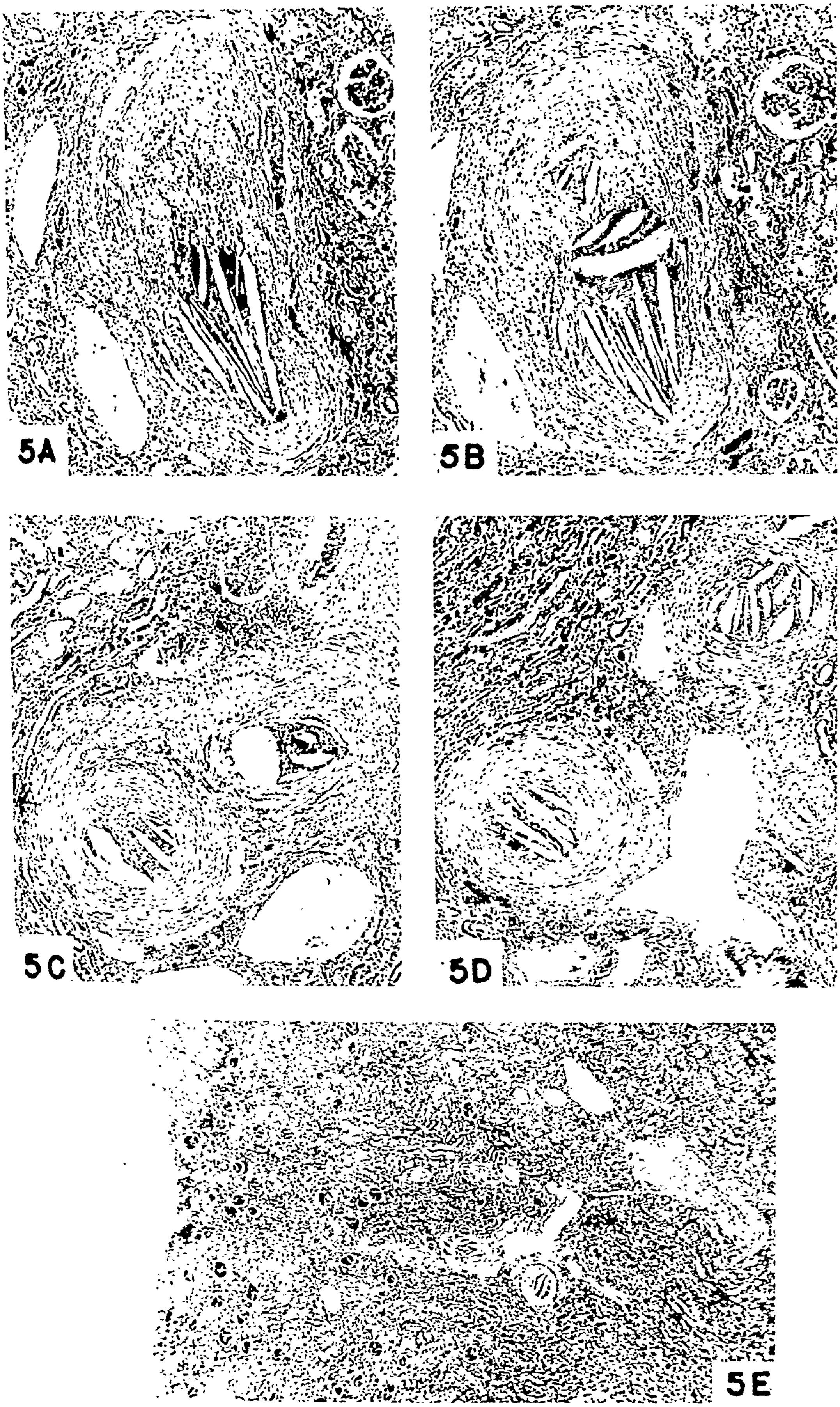


Flory

Emboli from Atheromatous Plaques

PLATE 93

FIG. 5. Case 1. Variability of the pattern of the occlusion in a medium-sized renal artery. In *Figure 5-A* the beginning of the bifurcation of the occluded vessel is shown. Many of the slit-like spaces are cholesterol crystal spaces, others are thin vascular spaces. *Figure 5-B* is the bifurcation of the vessel. In *Figure 5-C* the bifurcation is complete. The branches are partly filled with cholesterol crystal spaces. In *Figure 5-D* the vessels are farther apart. In the upper artery are many small cholesterol crystal spaces; in the lower vessel several large crystal spaces surrounded by giant cells. × 50. In *Figure 5-E* the area of cortical atrophy lying above the vessels shown in Figure 5-D is seen. × 20.



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Emboli from Atheromatous Plaques

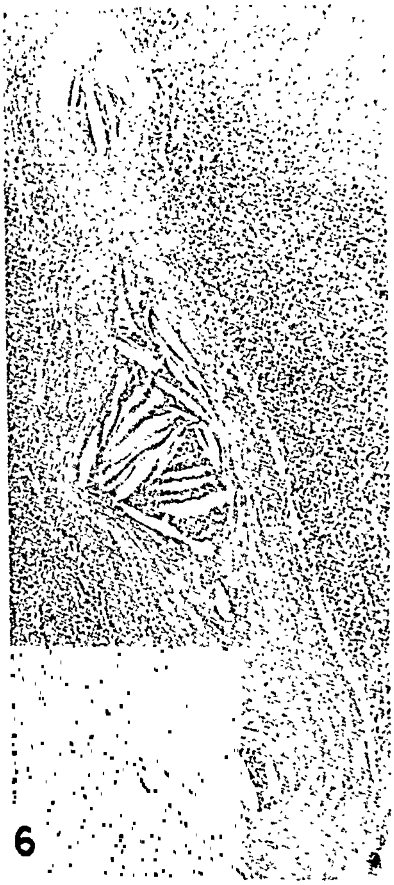
Plate 94

Fig. 6. Case 1. Three occluded splenic arteries. These arteries, the largest of which measured 540 μ in diameter, are probably all branches of a single larger vessel. They are filled with cholesterol crystal spaces. $\times$ 50.

Fig. 7. Case 1. An occluded small splenic artery. This artery is a branch of one of the vessels shown in Figure 6. It measures 94 μ in diameter and is filled with cholesterol crystal spaces surrounded by large foreign body giant cells.

Fig. 8. Case 1. A partially occluded small splenic artery. In this artery, which measured 84 μ in diameter, the cholesterol crystal spaces lie against the intimal surface of one side of the artery and are partly encased by giant cells. Several endothelium-lined vascular channels remain. $\times$ 640.

Fig. 9. Case 1. Partially occluded small splenic artery. The crystal spaces are attached to the surface of the intima of the artery and are almost encased by giant cells. This vessel measures 96 μ in diameter. $\times$ 160.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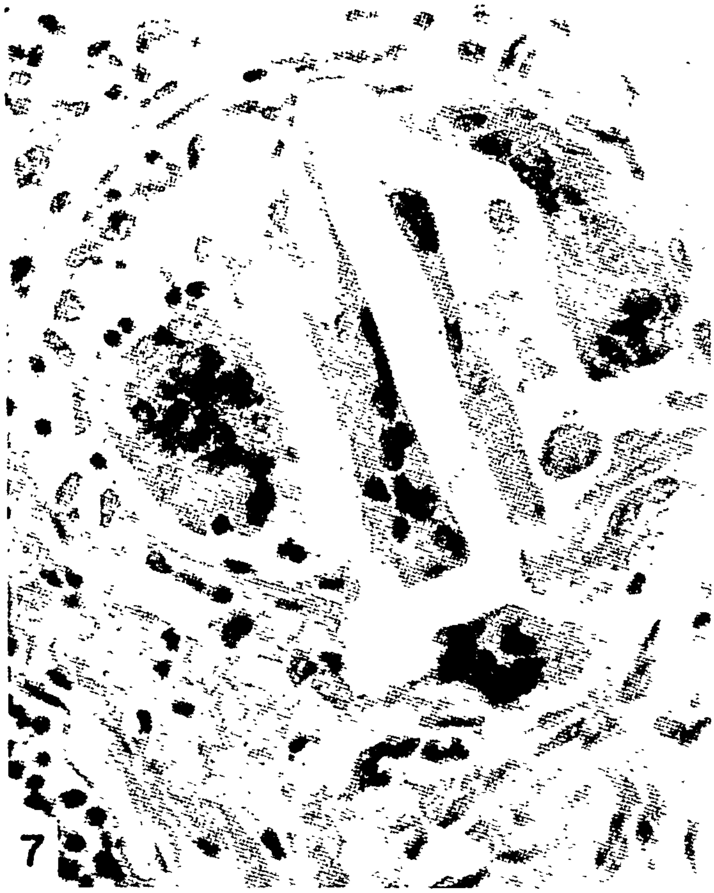
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Flory

Emboli from Atheromatous Plaques

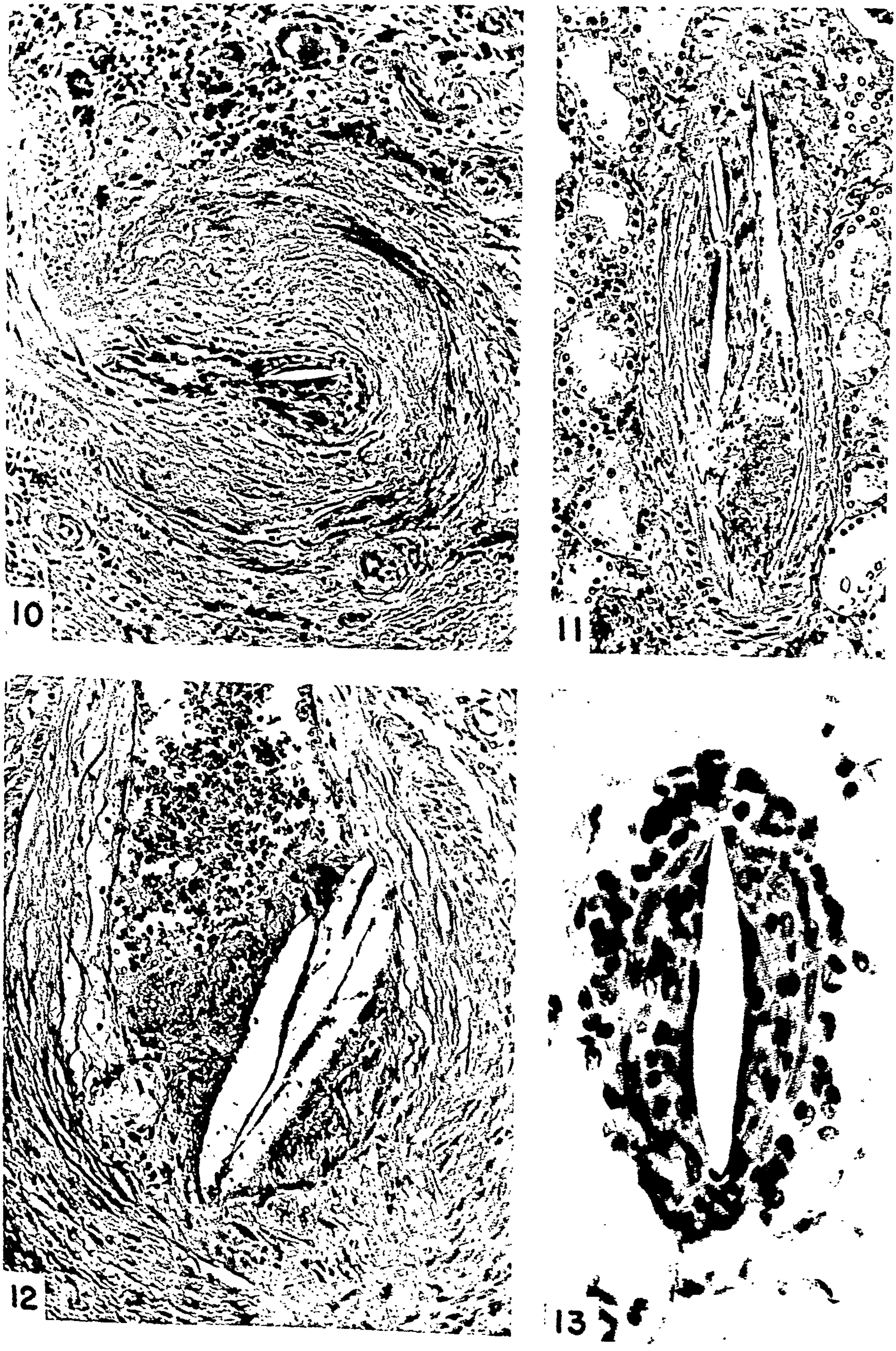
PLATE 95

FIG. 10. Case 2. Occluded renal artery. In the center of this vessel are two small cholesterol crystal spaces partly encased by giant cells. Several small vascular channels are present in the intimal tissue. × 160.

FIG. 11. Case 3. Partially occluded renal artery. Several large cholesterol crystal spaces are embedded in the hyperplastic intimal tissue of one wall of the vessel. A large lumen remains in the artery. This is probably an old lesion, and the vessel is almost completely recanalized. × 160.

FIG. 12. Case 5. Recent thrombus containing cholesterol crystal spaces in a medium-sized renal artery. The lumen of this longitudinally cut artery is occluded or partially occluded by a recent, partially organized thrombus in which are several large cholesterol crystal spaces. × 50.

FIG. 13. Cholesterol crystal space in artery of lung of rabbit. One week before death, this animal was injected intravenously with a suspension of cholesterol crystals from an atheroma of a human aorta. The large crystal space is surrounded by giant cells and intimal tissue. × 640.



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THE VAGINAL SMEAR IN DIAGNOSIS OF CARCINOMA OF THE UTERUS *

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A technic for the study of uterine malignancy by means of smears of vaginal secretions was first described by Papanicolaou in 1928.[1] The value of the technic has been demonstrated by Papanicolaou and Traut[2] in a beautifully illustrated monograph setting forth their experience on a large number of patients. Meigs, Graham, Fremont-Smith, Kapnick, and Rawson[3] have also emphasized the importance of this method as a routine diagnostic test.

The acceptability of a new method depends on two things: First, it must be shown to be at least as accurate as the methods already in use; and second, it must have some added advantage such as ease of performance. Both have been claimed for the vaginal smear method for diagnosis of uterine cancer, although Papanicolaou and Traut[2] and Meigs *et al.*[3] stress its place as an accessory method.

In this paper we are primarily interested in the practical aspects of the vaginal smear as a procedure for the routine pathologic laboratory. It is, after all, the practising pathologist who will determine the general usefulness of the test. The successful use of the vaginal smear, either for routine diagnosis or in the control of cancer, depends on an understanding of what can be expected from the method, and of the pitfalls in diagnosis.

The method is simple and if necessary may be performed by the patient. The only requirement concerning the patient is that there should be no interference with the vaginal tract, as from douching or examination, within 12 hours before the sample is taken. The secretions are aspirated from the fornix by a clean, *dry* glass pipette, about 8 inches

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long, provided with a bulb for suction, and are then expelled onto one or more clean, *dry* slides and thinly smeared in a manner similar to the making of blood smears. The slide is immediately placed in a bottle of 95 per cent alcohol, or equal parts of ether and 95 per cent alcohol. After 15 minutes' fixation, the smear is ready to be stained, but an indefinite period in the solution is not harmful. The smear may be dried after fixation and stained at any convenient time, but it is important to avoid drying before fixing. Papanicolaou's stain produces good nuclear detail and is satisfactory. It is important that all of these steps be carefully followed.

The reliability of the method has been reported as extraordinarily high. The figures given by Papanicolaou and Traut [2] and by Meigs and his co-workers [3] correspond closely. The following table shows the small percentage of discrepancy between the diagnoses made of cases by the smear method and by biopsy as reported by these writers.

	Papanicolaou and Traut	*Meigs et al.*
Carcinoma of Fundus		
Number of cases	53	12
Per cent positive by biopsy and negative by smear	9.3*	8.3
Carcinoma of Cervix		
Number of cases	127	46
Per cent positive by biopsy and negative by smear	1.6*	2.2

Meigs and co-workers [3] further reported that in a group of 63 cases, independent diagnoses made by two of the authors accorded perfectly, all the smears being clearly positive or negative, none "suspicious," and that "Of 153 negative cases positive smears were reported in 4—error 2.6 per cent." Papanicolaou and Traut [2] mentioned no false positive diagnoses in their series. Ayre [4] recently reported that 95 per cent of 40 patients with carcinoma of the uterus showed tumor cells in the vaginal smear. These are remarkable records indeed. Certainly material obtained for biopsy, unless the site is very carefully selected, does not give a true picture of the condition of the genital tract in a higher percentage of cases. In spite of this, however, those having the widest experience with the method are cautious in recommending it as a substitute for biopsy or curettage, and have emphasized the need of confirming the diagnosis made on the smear by examination of tissue before therapy is undertaken. This present restriction limits the advantages of the technic as a conclusive diagnostic procedure.

An unexpected and important feature of this method is the demon-

* Excluding 2 post-irradiation cases with negative smears in each group.

stration of carcinoma in its earliest stages before invasion of the stroma has occurred. Some of the patients with carcinoma *in situ* reported by Papanicolaou and Traut [2] had no symptoms and in some cases the biopsies were negative, but the diagnosis of carcinoma was confirmed after hysterectomy. Papanicolaou and Traut stated that their experience is not sufficient to speak uncompromisingly on the accuracy of the diagnosis of early carcinoma, but they nevertheless feel that the "vaginal smear is the only practical method thus far developed which is useful in revealing the very early carcinomatous processes, particularly those of the cervix." If subclinical carcinoma is demonstrable by smears, the method may be of incalculable aid in improving the curability of carcinoma of the uterus. This has been stressed by both Papanicolaou and Traut, and by Meigs.[3] But there are many considerations to be weighed in the diagnosis and treatment of early carcinoma and it may not be out of the way at this point to review them.

Early treatment, the present best hope for the control of cancer, now depends on both the awareness of the patient and the readiness of the physician to investigate symptoms thoroughly. The responsibility of the physician in the delay of treatment has been studied recently by Pack and Gallo [5] and by Harms, Plaut, and Oughterson.[6] In the first of these two reports the patient and physician were implicated in 18.0 per cent of the delays, the physician alone in 17.0 per cent of the delays. The figures in the second report are similar: 27.8 and 17.4 per cent respectively. Unfortunately, it does not seem likely that a physician who, from ignorance or neglect, does not thoroughly investigate a patient with uterine symptoms will make intelligent use of the vaginal smear method. Something certainly can be hoped for from education, both professional and lay, but this approach has its limits.[7]

Periodic examination of well patients is one way to procure early treatment of malignant neoplasms, and cancer control programs have been organized to this end. Only complete, careful statistical studies over a period of years will indicate the profitableness of this approach. Three recent reports of cancer control programs are interesting inasmuch as they vary in organization and special interest. The Strang Cancer Prevention Clinic for Women in New York City organized by L'Esperance [8] has given complete physical examinations and followed patients at intervals of 6 or 12 months. Among 1,103 patients, not all symptomless, examined in 5½ years, there were 7.6 per cent with malignant tumors, the majority of them being early. In a similar clinic at the Memorial Hospital, 1.5 per cent with malignant tumors were found among 263 patients who had no symptoms.[8]

Macfarlane [9] reported the results of periodic pelvic examinations on

well women. Of 1,321 women who were given a single pelvic examination, many returned for further examinations and 255 of them were seen twice yearly for 5 years. Altogether, four carcinomas, all without symptoms, were found as a result of 8,822 examinations over a 5-year period. Three of these carcinomas were found at the first examination, the other one in the fifth year of examinations.

In a study of early carcinoma of the stomach, St. John, Swenson, and Harvey [10] examined fluoroscopically 2,413 patients who had no gastric symptoms. Those who showed abnormalities were followed further, making a total of 2,923 examinations. As a result of this data, partial resection of the stomach was performed on 8 patients. In 5 patients benign lesions were found; but in 3 the lesions were malignant and in 2 consisted of extremely small, early tumors, a carcinoma and a lymphosarcoma; in the third patient there were two malignant neoplasms with a large fungating growth. Metastases were not found in these 3 patients at operation, although the patient with lymphosarcoma died later of generalized disease.

Such studies as these present new problems as to both diagnosis and treatment of early carcinoma, which in view of our limited knowledge require a conservative attitude toward this aspect of malignancy. For example, in the cervix the differentiation of precancerous change, carcinoma *in situ,* and early invasive carcinoma may be a matter of individual judgment in the interpretation of tissue taken for biopsy and impossible from a smear. There is also some evidence that carcinoma *in situ* of the cervix is a sluggish process comparable to Bowen's disease of the skin.[11] Since many of the patients are in the child-bearing age and some very young, the diagnosis and treatment of early carcinoma should not be lightly undertaken.

Malignant growth is customarily recognized by a number of alterations of tissue as much as by specific changes in single cells. The pattern and boundaries of a growth are generally considered to be as reliable a guide in determining malignancy as the changes within cells. Frequently hyperplastic or metaplastic cells are difficult to distinguish from neoplastic cells in the absence of tissue relationships. It is therefore necessary to know the benign pathologic variations of cells in question before attempting to diagnose their malignant changes.

The characteristics of the malignant cell have received a great deal of study. Although changes in the cytoplasm of malignant cells have been observed, such as increase in size and density, increase in number, and decrease in size of mitochondria,[12] the visible changes most often described involve the nucleus. The nucleus of the malignant cell tends to be larger than that of the normal cell, especially in relation to

the volume of the cell. The nuclear membrane is often thicker.[12] A rather striking alteration is the increase in the granular material of the nucleoplasm. The large size of one or more nucleoli has been emphasized by most observers of malignant cells, possibly because this is more easily gauged than some of the other aberrations. MacCarty, using fresh tissues removed at operation, first stressed the relationship of nucleolus and nucleus of the malignant as compared to the normal cell.[13-16] Others, using various methods of fixation, have reached the same conclusions as to the relative increase in size of the nucleolus of malignant cells[17-19] and Lewis[12, 20] observed more than the usual amount of nuclear material in malignant cells in tissue culture. Although the visible change in the malignant cell is, to a certain degree, measurable, this should not be taken to indicate that the various modifications are certainly pathognomonic in themselves. Lewis[12] pointed out that malignant cells in culture vary considerably from one to another: "The size, number, position of the nucleoli all vary more or less among cells in the same culture. No two cells are exactly alike." Furthermore, practically all differences noted between normal and abnormal cells are subject to modifications. Our knowledge is yet too fragmentary and inadequate to make a summing up of characteristics more than provisional.[12]

Nevertheless, the observed modifications from the norm have proved to be a practical distinction in diagnosis of malignant cells; for example, the relative size of nucleus and nucleolus as determined by plane or volumetric measurements in normal and pathologic tissue. Foot[17] found the ratio of the diameter of the nucleus to that of the nucleolus to be higher in malignant cells than in normal cells. In malignant cells, the average ratio of nucleolus to nucleus is 0.2 to 0.4, whereas in the normal cell it is below 0.2. MacCarty found that the ratio of nucleolar area to nuclear area varied from 1:5 to 1:17 for malignant cells and 1:13 to 1:45 for nonmalignant cells.[15]

Guttman and Halpern[21] made a great many measurements on benign and malignant tumors and on normal tissues and concluded that the quantitative differences between nuclear-nucleolar volume ratios of normal tissue, hyperplastic tissue, benign and malignant tumors were not significant and that such measurements should be used only as an aid and not as a decisive factor in determining whether or not a tumor cell is malignant. These authors rightly pointed out that nucleoli may be larger in simple hyperplastic tissues than in malignant tissues and that in some malignant tumors the nucleoli may be as small as in normal cells. This serves to emphasize what every pathologist is aware of, namely, that the source and condition of the cells or tissue examined

are important factors and sometimes are essential considerations in evaluating cells. Radiation reaction of tissues may be taken as an example. Both fibroblasts and epithelial cells in irradiated tissue may acquire all the morphologic features of malignant cells and yet remain to all intents and purposes benign. Comparison of cells in malignant and benign growths with the normal cells of their tissue of origin is manifestly more enlightening than comparison of heterogeneous normal tissues and tumors. Lewis [20] observed in tissue cultures that each type of malignant cell usually has one or more characteristics which differentiate it from normal cells. Most pathologists would probably agree with MacCarty's [15] statement: "The cancer cell may not always be distinguished from a normal regenerating cell, but this can be done frequently because there is a difference in volume-relationship between nucleolus, nucleus, and the whole cell in reparative regenerative cells and malignant regenerative cells."

The practicability of differentiation of solitary malignant cells from isolated normal cells in exudates from body cavities was demonstrated long ago, but the accuracy of diagnosis has never been remarkable, probably due in large part to the sample. The figures reported by Foot [17] are instructive. A group of selected cases, checked by biopsy, autopsy, or operation, were examined three times, and the percentage of accuracy given for each of the examinations. In specimens of abdominal fluid in which tumor was present, the percentage of accuracy for the three examinations was 73, 60, and 72, giving an average accuracy of 68 per cent. Anyone interested in this subject should read Foot's review and study Quensel's [18, 19] beautiful figures. Schlesinger's [22] study is also very useful.

In the vaginal smear the disadvantage of the sampling method is not apparent. There is usually a plethora of exfoliated cells since the whole epithelial surface of the genital tract may be represented. The diagnostic value of the smear depends on the greater tendency of malignant cells to be shed as compared to normal cells, as pointed out by Papanicolaou and Traut.[2] This loss of cohesiveness of the malignant cells has been measured by Coman,[23] who found that cells of carcinoma of the cervix could be separated with less than one-sixth of the force required to separate normal epithelial cells of the cervix. In spite of this there are usually relatively few carcinoma cells in a smear as compared to other cells, due to such factors as: (1) the ulceration, necrosis, and infection of the larger growths with decreased viability of the neoplastic cells, and also the masking of cells by the numerous polymorphonuclear leukocytes; (2) the greater fragility of malignant cells; (3) the small area of exposed tumor as compared to the whole surface of the genital tract. Variations in these factors explain why carcinoma cells may be

few or absent in the smears when large ulcerated growths are present, while appreciable numbers of well differentiated malignant cells may be scattered through the smear when the carcinoma is still *in situ* and not detectable on clinical examination.

Many points of practical importance in diagnosis of vaginal smears should be more explicitly demonstrated from detailed data on individual cases:

(a) What is the correspondence of a smear with (1) simultaneous clinical observation, and (2) simultaneous biopsy?

(b) In how many cases does a single smear suffice for the diagnosis?

(c) To what degree are consecutive smears consistent?

(d) How time-consuming is the examination of the smear?

(e) Can precancerous conditions be distinguished from cancer?

(f) How often is subclinical carcinoma, either as a primary condition or as recurrence after operation or radiation, detected by the smear?

(g) Is extensive cytologic experience necessary for gaining proficiency in diagnosis?

This present study is based on vaginal smears from patients of the out-patient gynecologic clinic of the New England Deaconess Hospital, from patients of the Pondville Hospital, of Westfield Sanatorium, of the Lahey Clinic, and from private patients of physicians using the State Tumor Diagnosis Service. We are indebted to Dr. J. V. Meigs, Chief of the Gynecological Clinic of the Palmer Memorial unit of the New England Deaconess Hospital, for the majority of the smears. The patients were in a sense selected in that the smear was not a routine procedure for all the patients in any of the clinics. Smears were made from some patients with no evidence of pelvic disease.

During the period of 9½ months from February through November 15, 1944, we examined 341 smears from 233 patients. The diagnosis of these smears was undertaken without any preliminary study and formed a small fraction of the routine work of the laboratories so that prolonged study of individual smears for diagnosis was not feasible. All reports were made without knowledge of the patients' histories or of the clinical diagnoses. Not until some time had passed did we attempt to study the group as a whole, and correlate the diagnoses on the smears with the clinical and pathologic findings. For this report, we have reviewed all of the smears.

The stain recommended by Papanicolaou and Traut [2] was used for nearly all of the smears.* The others were stained with hematoxylin and eosin and were quite satisfactory. One of the most important

* This stain was developed by G. N. Papanicolaou. (A new procedure for staining vaginal smears. *Science*, 1942, 95, 438–439.)

factors is the thinness of the smear. Too often only a small part of the secretions can be examined because of the density of the material. This can be easily overcome by spreading the material on more than one slide in a manner similar to making blood smears.

We shall review briefly some of the salient histologic features of the smears and lastly present our cases.

The normal and pathologic cells found in smears have been clearly described and beautifully illustrated in the monograph of Papanicolaou and Traut.[2] We shall, therefore, review only those histologic features of the smears which we have found of special importance. The most numerous normal cells are the squamous cells of the exocervix and vagina which vary in appearance according to their derivation from the inner (basal) or outer (superficial) part of the epithelium. The superficial cells are large, tend to be polyhedral, and the nuclei are very small in proportion to the cytoplasm. The "basal" cells are smaller, rounded, with a slightly larger nucleus than the superficial cell. Cells of both types undergo minor changes in structure under varying physiologic conditions, such as menstruation, pregnancy, and the menopause. While it is useful to understand these variations, an intimate knowledge of them is not essential for the study of malignancy, as none of them would cause any difficulty in differentiation from malignant cells. On the other hand, atrophy or infection of the cervix and vagina may produce pathologic changes of the "basal" cells closely simulating malignancy. Endometrial and endocervical cells are not commonly seen except during menstruation, when they occur singly and in clumps. They are minute in comparison with squamous cells, are oval, oblong, or pointed. The nucleus is finely powdered and the cytoplasm appears as a faintly colored fringe or tail at either end.

The various forms of malignant cells are too numerous to be described. A close study of the figures in the monograph by Papanicolaou and Traut [2] is most useful, especially after some experience with the smears. As would be expected, we have found nuclear changes to be the most reliable criteria of malignancy. A nucleus which is larger than normal in proportion to its cytoplasm should arouse suspicion, and still more should a relatively large nucleus with unusual appearance or arrangement of chromatin. The nucleus of the malignant cells we have seen in smears may be pale and finely powdered with granules; occasionally the chromatin is coarse, scattered, sometimes closely attached to the nuclear membrane. The nucleoli are often large, but stray granular fragments overlying the nucleus may suggest an enlarged nucleolus. In some malignant cells the nucleus is agranular and takes a homogeneous deep basophilic stain, but these cells usually

show some evidence of degeneration. In many of the malignant cells the centriole is distinctly visible. Mitotic figures are so seldom found that their significance is negligible. We have seen mitotic figures in smears in which the cells were atypical but not clearly malignant. Naked nuclei are frequent and often confusing but should not be taken seriously unless they are distinctly abnormal in structure. The staining reaction of the cytoplasm of the malignant cells is variable and has no practical significance. Striking variation in size of malignant cells has been found in relatively few cases and this characteristic may not be of great value inasmuch as nonmalignant normal and pathological cells are so conspicuously variable in this respect. However, as Papanicolaou points out, within a group of cells anisocytosis is quite significant. Variation in size may be greater in epidermoid carcinoma of low or moderate malignancy than in the less differentiated forms.[2]

The arrangement of carcinoma cells in clumps of more than three or four has not been a conspicuous feature in our preparations, or perhaps it would be more correct to say that we have had difficulty in evaluating the large sheets of cells which sometimes appear in smears. The masses are frequently so dense that no clear conception of the individual cells is possible, and the variation in size of these clumped cells is difficult to gauge. Papanicolaou and Traut [2] pointed out that groups of epithelial cells containing polymorphonuclear leukocytes are rarely found in nonmalignant conditions and should arouse suspicion of carcinoma, although this is not an absolutely pathognomonic change. In our experience, sheets of cells have been seen most often in cervicitis, vaginitis, and radiation reaction.

Single malignant cells do not always carry distinctive features indicating their origin. Those seen in smears often bear little resemblance to the normal cell types of the uterus seen in section, and the classification of the type of carcinoma is more difficult than the recognition of malignant cells. Papanicolaou and Traut [2] stated that "The grade I and II squamous carcinomas of the cervix . . . offer the least difficulty . . . although they are capable of producing a much greater variety of morphological variation," whereas, in the grade III squamous carcinomas many of the cells are less readily recognized as malignant and there are fewer malignant cells present. They also stated that many of the undifferentiated squamous carcinomas may produce cells difficult to distinguish from undifferentiated adenocarcinomas of the cervix. In differentiated carcinoma of the endometrium, the cells may not vary greatly from normal cells, but the presence of endometrial cells, except during menstruation, is abnormal and an unusual number or clumping of endometrial cells is strong evidence of adenocarcinoma.

However, in sections, the cells of an adenocarcinoma may not vary appreciably from those of hyperplasia. In smears, many of the cells are vacuolated with eccentric nuclei of signet ring type. In undifferentiated adenocarcinoma the cells may be very small with dark basophilic nuclei and only fine tails of cytoplasm.

The main points of our results will be found in the accompanying tables. Since it is our aim to evaluate the smear as a routine diagnostic procedure, we have given in each case the initial as well as the final diagnosis. The last diagnosis represents judgment based on our total experience to date and like the first was made without knowledge of the clinical or pathologic findings. Many of the smears were technically unsuitable. Some smears were entirely unsatisfactory and many others nearly so. We graded the smears as good, fair, poor, or unsatisfactory (one-fourth of the smears were poor, and 16 others were unsatisfactory for diagnosis and were recorded as negative for statistical purposes). Since we wish to present our experiences entirely unbiased, we concluded that the fairest picture would be obtained by including all of the smears in our tables, although better smears would undoubtedly have eliminated some discrepancies. In two instances positive smears represented material aspirated directly from carcinomatous ulcers rather than from secretions.

TABLE I

General Summary of Cases

	Total	Total nonmalignant	Malignant†			
			Total malignant	Nonradiated	Radiated	
					Less than 3 months	More than 3 months
Cases	233	101	132	33	43	56
Smears*	341	123	218	45	75	98

* The numbers indicate smears taken at different times. Duplicate smears taken at the same time are counted as a single smear.

† In all but 4 of our cases classified as malignant there were positive biopsies, and clinically these 4 were unquestionably carcinoma.

In Table I the sum of our material is outlined. All but 4 of our cases classified as malignant had been determined to be positive by biopsy at one time or another. These 4 patients had gross tumors of unquestioned malignancy. Only 33 of the malignant cases (45 smears) were not radiated. The post-radiation cases are grouped as recent or remote depending on whether there had been a biopsy within 6 months of the time the smear was taken. The 86 recent cases (159 smears) were mainly carcinoma of the cervix treated by radiation. Thirteen

of these were recurrent carcinoma of the cervix 1 to 4 years after radiation. Sixteen of the recent cases (31 smears) were of adenocarcinoma or adenoacanthoma. The 46 remote cases (59 smears) had been examined by biopsy anywhere from 7 months to 11 years before the smear was taken. These 46 patients, with one exception, had been radiated. Twenty-one of them had remained without evidence of disease for 2 to 11 years.

The types of lesions of malignant and nonmalignant cases are shown in Table II. The cases classified as nonmalignant were considered clinically as benign, but only 36 of them were examined by biopsy. We have arbitrarily included in this group several exceptional cases: 3 patients who had had a hysterectomy for adenocarcinoma of the fundus 2 to 4 years before the smear was taken and since had been free from disease; 1 case with localized carcinoma of the vulva; 9 cases with incomplete data; 6 cases (9 smears) without any clinical data.

Table III shows the distribution of the revised diagnoses on the radiated and nonradiated group. The smears in which the first and final diagnosis did not correspond exactly are here tabulated in sequences of 50, to show the effect of experience. There are 10 other smears not tabulated, 9 of them from radiated patients, which we have not been able to interpret. Although there are nearly the same number of smears in the nonradiated and radiated groups (168 to 173), the majority of the changes in diagnosis were on smears from radiated patients. These smears with a change in diagnosis on the final review are given again with the complete data on the patients in the three following tables.

In Tables IV, V, and VI are cases in which the diagnoses from the smears did not closely correspond with the other data. These cases are grouped as follows:

Table IV—Clinically benign and doubtful cases
Table V—Malignant cases not radiated
Table VI—Malignant cases radiated

The clinical and pathologic diagnoses are those made *at the time* the smear was taken or within a week or 10 days before the smear was taken, provided no treatment intervened. The column headed "Type of Carcinoma" is included for the benefit of those smears without a simultaneous biopsy and gives the pathologic diagnosis made at an earlier or later date. All but a few of the pathologic diagnoses were made at another laboratory. We have chosen this rather elaborate form of presentation of cases as the clearest way to correlate all of the information on a single patient, and to show the type of case in which doubt and error are most likely to occur. These three tables present

TABLE II
Types of Lesions

	Benign					Malignant								
	Cervicitis or vaginitis		Other		Total benign	Epidermoid carcinoma					Adeno-carcinoma	Other‡	Total malignant	Grand total
	Biopsy	No biopsy	Biopsy	No biopsy*		I	II	III	Not graded†	Total				
Cases	18	5	13	65	101	8	23	33	29	93	19	20	132	233
Smears	23	5	18	77	123	17	36	51	49	153	35	30	218	341

* This includes incomplete cases.
† Undifferentiated carcinoma and carcinoma insufficient for classification.
‡ Includes 4 unbiopsied cases.

every case in which there is any inconsistency in the data. The discrepancies in these tables are of two kinds: (1) Changes in the diagnoses of the smears. This is an elaboration of the data in Table III. (2) Discrepancies between the final diagnosis on the smear and the clinical and pathologic diagnoses. (a) Some of these differences are probably due to errors of interpretation, especially to failure to recognize the malignant cells, but definite proof of error is lacking. (b) In a few instances the patient was clinically free of disease but biopsy and smear proved its presence.

In the group of 123 smears from 101 patients without clinical or pathologic evidence of malignant disease in the genital tract, there is one false positive diagnosis, and this an initial error. Table IV includes this case with 8 other doubtful cases. The definite false positive smear is no. 726, case 30. This was taken from a woman, 82 years old. Three biopsies showed chronic cervicitis and 3 subsequent smears were called negative. Our final diagnosis on this smear is negative.

Table V presents 12 malignant, nonradiated cases lacking conformity in diagnoses of smear and clinical and pathologic data. Eight cases are included because of a doubtful initial diagnosis or a final change in diagnosis. One case, no. 96, is also in Table VI since all but the first smear were taken during radiation. Cases 18 and 31 will be discussed later in connection with early carcinoma.

TABLE III

Changes in Interpretation of Smears Arranged in Chronologic Series of 50 Smears Each

Changed from positive to negative	1st	Radiated	2nd	Radiated	3rd	Radiated	4th	Radiated	5th	Radiated	6th	Radiated	Total not radiated	Total radiated	Grand Total
Not radiated	1														
During radiation															
1 to 6 mos. post-radiation		1													
6 mos. to yrs. post-radiation		3		1											
Totals													1	5	6
Changed from negative to positive															
Not radiated									1						
During radiation				2		1		1				1			
1 to 6 mos. post-radiation						1		1		1					
6 mos. to yrs. post-radiation				1		1		1		1		1			
Totals													1	13	14

TABLE III (*Continued*)

Changed from questionable to negative	1st	Radiated	2nd	Radiated	3rd	Radiated	4th	Radiated	5th	Radiated	6th	Radiated	Total not radiated	Total radiated	Grand Total
Not radiated	1		1		1				1						
During radiation						1									
1 to 6 mos. post-radiation		·2						1							
6 mos. to yrs. post-radiation		4		2		1		1		1					
Totals													4	13	17
Changed from questionable to positive															
Not radiated	1		1		1		1		3						
During radiation				2		2						2			
1 to 6 mos. post-radiation		2										1			
6 mos. to yrs. post-radiation															
Totals													7	9	16

The cases which were radiated are more difficult to analyze. In many of them the interval between the biopsy and the smear was too long for correlation. Smears taken during radiation were frequently unsatisfactory because of degeneration. The greatest difficulty, however, arises from an uncertainty of interpretation of the cases in which radiation reaction is present. In Table VI are those radiated cases in

TABLE IV

Cases Which Were Not Malignant from Clinical or Pathologic Evidence in Which Smear Diagnosis Did Not Conform to Other Data

Case no.	Smear no.	First diagnosis	Final diagnosis	Time between smears	Clinical data	
					Clinical diagnosis	Diagnosis by biopsy
30	726	+	–		Cervicitis	Cervicitis
	892	–	–	12 days		
	1157	–	–	3 weeks		
	2437	–	–	4 months		
47	1002	Suggests low-grade carcinoma; not definite	–		Negative	
	1159	–	–	2 weeks		
86	1737	Cells suggesting tumor present	–		Chronic cervicitis	Chronic cervicitis
	2041	–	–	3 weeks		
149	2599	+(?)	–		Cervicitis	Cervicitis
189	3033	–	–		Endometriosis	
	3371	+	+	1 month	Endometriosis	Early epidermoid carcinoma(?)
202	3196	+	+		Carcinoma of cervix(?)	Negative
206	3200	+	+		Uncertain	Negative
214	3208	Scattered carcinoma cells	+		Pelvic tumor	Negative
229	3370	+	+		Uncertain	Hyperplasia of endometrium

which the first or last diagnosis on the smear differed from the other data. These discrepancies are not necessarily errors of the method, as study of the table will show. They represent also deficiencies in the biopsy procedures and in clinical data.

Thus far we have considered the smear chiefly as subordinate evidence subject to correction or confirmation by biopsy. In some cases the positive smear may be the first, or even the only, preoperative evidence of carcinoma, and it is in lesions of this sort that the smear may have the greatest importance as an accessory method of diagnosis.

TABLE V

Discrepancies Between Smear and Other Diagnoses on Malignant, Not Radiated, Cases

Case no.	Smear no.	First diagnosis	Final diagnosis	Time between smears	Clinical data		
					Clinical diagnosis	Biopsy diagnosis	Type of carcinoma
18	458	−	−		Negative	Very early carcinoma	Very early carcinoma
	1217	−	−	2 mos.			
	1865	−	−	2½ mos.			
	2762	−	−	3 mos.			
	3538	−	−	2 mos.			
27	627	One cell suggests carcinoma	−		Uncertain	Undifferentiated carcinoma, endometrium	Undifferentiated carcinoma, endometrium
28	628	One cell suggests carcinoma	+		Carcinoma	Carcinoma	Epidermoid carcinoma, grade III
31	728	+	+		Negative	Carcinoma *in situ*	Carcinoma *in situ*
50	1079	One carcinoma cell(?)	+		Carcinoma	Carcinoma	Epidermoid carcinoma, grade III
	1139	+	+	1 week	Carcinoma		
	3452	−	−	7 mos.	Carcinoma	Epidermoid carcinoma, grade III	
81	1693	−	+(?)		Carcinoma	Epidermoid carcinoma, grade I	Epidermoid carcinoma, grade I
	2331*	−	−	2 mos.			

TABLE V (*Continued*)

Case no.	Smear no.	First diagnosis	Final diagnosis	Time between smears	Clinical data		
					Clinical diagnosis	Biopsy diagnosis	Type of carcinoma
96	2040	−	+		Senile vaginitis	Carcinoma	Adenocarcinoma
	2296	−	+	3 weeks			
	2485	+	+	3 weeks			
	3142	−	−	2 months		Carcinoma	
103	2187	Suggests carcinoma(?)	+		Carcinoma	Carcinoma	Adenocarcinoma
	3019	Suggests carcinoma(?)	+	2½ months			
146	2587	Few carcinoma cells(?)	+		Carcinoma	Epidermoid carcinoma	Epidermoid carcinoma, grade II
173	2842	−	+		Carcinoma	Adenocarcinoma	Adenocarcinoma
	3076*	+	+	3 weeks			
174	2869	Probably adenocarcinoma	+		Senile vaginitis	Carcinoma	Adenocarcinoma
220	3254	−	−		Stage 3 lesion	Epidermoid carcinoma, grade III(?)	Epidermoid carcinoma, grade III

* These smears were taken during, or immediately after, radiation.

TABLE VI

Discrepancies Between Diagnoses from Smear and Other Diagnoses on Cases of Post-Radiation Carcinoma

Case no.	Smear no.	First diagnosis	Final diagnosis	Time between smears	Clinical data: Simultaneous clinical diagnosis	Clinical data: Simultaneous biopsy diagnosis	Clinical data: Time between radiation and smears	Radiation reaction	Type of carcinoma
1	171	−	−		Carcinoma	Epidermoid carcinoma, grade II	1 month	−	Epidermoid carcinoma, grade II
	893	−	+(?)	2 months	Uncertain		3 months	−	
	1866	−	−	3 months	Uncertain		6 months	−	
	2841	+	+	3 months	Carcinoma		9 months	+	
5	209	A few carcinoma cells	−		No tumor		7 years	+	Adenoacanthoma
	465	Two carcinoma cells	−	3 weeks				+	
	727	A few small carcinoma cells	−	3 weeks				+	
	1445	−	−	2 months				+	
	2439	−	−	3 months				+	
6	210	Rare cells suggesting carcinoma	−		Negative		4 months	+	Adenocarcinoma
	461	Carcinoma cells(?)	−	3 weeks			5 months	+	
	1297	Probably carcinoma	?	2½ months			8 months	+	
	2274	−	−	3 months			11 months	+	
10	296	−	−		Carcinoma		9 months	?	Adenocarcinoma
	462	+	+	2 weeks			9 months	?	
	1158	Suggests carcinoma(?)	+	2 months		Adenocarcinoma	11 months	−	
	2196	+	+	3½ months	Carcinoma		14 months	−	
	3257	+	+	3 months	Carcinoma		2 months	−	

Table VI—*continued*

Case no.	Smear no.	First diagnosis	Final diagnosis	Time between smears	Clinical data: Simultaneous clinical diagnosis	Clinical data: Simultaneous biopsy diagnosis	Clinical data: Time between radiation and smears	Radiation reaction	Type of carcinoma
11	297	+	+		Negative	Negative	8 months	+	Carcinoma
12	352	Carcinoma(?)	+		Carcinoma	Carcinoma	During radiation	?	Adenocarcinoma
	806	Carcinoma(?)	+	5 weeks			At end of radiation	−	
17	370	A few cells suggesting well differentiated carcinoma	−		Negative		3 years	+	Epidermoid carcinoma, grade III
21	463	+	+				4 years	?	Epidermoid carcinoma, grade III
	1078	One malignant cell(?)	+	2 months		Carcinoma with radiation reaction	During radiation	?	
	1583	−	+(?)	2 months		Rare cells suggesting carcinoma	3 months	?	
	2426	−	+	3 months	Negative	Radiation reaction with a few carcinoma cells	5 months	+	
22	464	Sheets of carcinoma cells	−		Abdominal mass		1 year	+	Adenocarcinoma
	808	A few cells suggest carcinoma	−	1 month	No vaginal or uterine tumor		13 months	?	
24	407	−	−		Carcinoma		2 months	−	Epidermoid carcinoma, grade III

TABLE VI—*continued*

Case no.	Smear no.	First diagnosis	Final diagnosis	Time between smears	Clinical data: Simultaneous clinical diagnosis	Clinical data: Simultaneous biopsy diagnosis	Clinical data: Time between radiation and smears	Radiation reaction	Type of carcinoma
26	626	Scattered cells suggesting well differentiated carcinoma	–		Negative		2 years	?	Epidermoid carcinoma, grade I
	1577	–	–	3 months	Negative		2 years	?	
	2560	A few cells suggest carcinoma	–	3 months	Negative			+	
34	809	+	+		Carcinoma		4 years	+	Epidermoid carcinoma, grade III
	2484	–	+	5 months	Extensive disease			+	
35	810	+	+		Negative		8 months	+	Epidermoid carcinoma, grade III
	3079	Atypical cells; no definite carcinoma	+	7 months	Ulcer, radiation reaction(?), carcinoma(?)	–	2 months	+	
38	894	Low grade carcinoma	–		Negative		3 months	+	Epidermoid carcinoma, grade II
40	896	–	+		Carcinoma	Epidermoid carcinoma, grade II		–	Epidermoid carcinoma, grade II
	1172	–	+	3 weeks	Carcinoma	Epidermoid carcinoma, grade II	At time of radiation	?	
	1579	–	–	6 weeks	Carcinoma		1 month	–	
	2909	–	–	4 months	Carcinoma	Wertheim's operation; negative	5 months	–	

TABLE VI—*continued*

Case no.	Smear no.	First diagnosis	Final diagnosis	Time between smears	Clinical data: Simultaneous clinical diagnosis	Clinical data: Simultaneous biopsy diagnosis	Clinical data: Time between radiation and smears	Radiation reaction	Type of carcinoma
46	1001	Low grade carcinoma	—		Negative		5 years	+	Epidermoid carcinoma, grade I
	1137	—	—	2 weeks				+	
	1215	—	—	1 week				?	
	2189	—	—	3 months				—	
53	1140	Suggestive carcinoma cells	—		Disease present		2 months	—	Epidermoid carcinoma, grade II
	2190	—	—	3 months			5 months	—	
56	1213	—	—		Negative		4 months	—	Epidermoid carcinoma, grade I
	2195	A few cells suggesting carcinoma, not definite	—	3 months			7 months	—	
	2840	—	—	2 months			8 months	+	
58	1216	—	—		Extensive disease		4 years	+	Epidermoid carcinoma
	3530	Atypical cells degenerative, radiation reaction(?), carcinoma(?)	+(?)	7 months	Negative	—	2 months		
60	1219	Suggests carcinoma	—				2 years	+	Carcinoma of cervix
	2191	—	—	3 months				+	

TABLE VI—*continued*

Case no.	Smear no.	First diagnosis	Final diagnosis	Time between smears	Clinical data: Simultaneous clinical diagnosis	Clinical data: Simultaneous biopsy diagnosis	Clinical data: Time between radiation and smears	Radiation reaction	Type of carcinoma
65	1359	−	+		Carcinoma	Epidermoid carcinoma, grade III	5 months	?	Epidermoid carcinoma, grade III
	2188	+	+	2½ months		Radiation reaction with carcinoma	At completion of radiation	−	
69	1363	Low grade carcinoma(?)	−		Negative		1 year	−	Epidermoid carcinoma
	2987	−	−	5 months			5 months	−	
70	1425	−	−		Carcinoma			−	Unknown
	1466	−	−	4 days			During radiation	−	
76	1535	−	+		Carcinoma	Epidermoid carcinoma ungraded with radiation reaction of stroma	2 months	−	Epidermoid carcinoma
	1582	2 cells suggest carcinoma	?	1 week	Carcinoma of cervix	Epidermoid carcinoma with radiation reaction of stroma	2 months	?	
82	*1694*	*Suggests carcinoma*	−		*Negative*		*8 years*	+	*Epidermoid carcinoma, grade III*
85	1736	Suggests carcinoma	−		Carcinoma of vagina	Epidermoid carcinoma, grade I	During radiation	−	Epidermoid carcinoma, grade I
	1739	Suggests carcinoma	+	Simultaneous	Carcinoma		During radiation	−	
	1811	−	−	3 days	Carcinoma		During radiation	−	
	1902	−	−	1 week	Carcinoma		During radiation	−	

TABLE VI—*continued*

Case no.	Smear no.	First diagnosis	Final diagnosis	Time between smears	Clinical data: Simultaneous clinical diagnosis	Clinical data: Simultaneous biopsy diagnosis	Clinical data: Time between radiation and smears	Radiation reaction	Type of carcinoma
94	1966	−	+(?)		Recurrence(?)		13 months	−	Epidermoid carcinoma, grade III
96	2040	−	+		Senile vaginitis	Carcinoma		−	Adenocarcinoma
	2296	−	+	3 weeks			During radiation	−	
	2485	+	+	2 weeks			1 month	+	
	3142	−	−	2 months		Carcinoma	3 months	−	
103	2187	Suggests carcinoma(?)	+		Carcinoma	Carcinoma	Before radiation		Adenocarcinoma
	3019	Suggests carcinoma(?)	+	2½ months			At end of radiation	+	
107	2222	+	+					−	Epidermoid carcinoma
	2241	−	−	2 days		Epidermoid carcinoma with radiation reaction	After radiation	−	
109	2299	−	−		Carcinoma	Radiation reaction with small focus of carcinoma	During radiation	−	Carcinoma
	3342	+(?)	+(?)	3 months	Carcinoma	−	3 months	−	
122	2413	−	+		Negative	Carcinoma	2 years	−	Epidermoid carcinoma, grade III
130	2422	+	+		Negative	Negative	4 months	−	Adenoacanthoma
142	2472	Probably carcinoma(?)	+			Carcinoma	During radiation		Epidermoid carcinoma, grade III

TABLE VI—*continued*

Case no.	Smear no.	First diagnosis	Final diagnosis	Time between smears	Clinical data: Simultaneous clinical diagnosis	Clinical data: Simultaneous biopsy diagnosis	Clinical data: Time between radiation and smears	Radiation reaction	Type of carcinoma
145	2564	−	+		Uncertain		4 months	−	Epidermoid carcinoma
	2699	+	+	1 week		Radiation reaction with two carcinoma cells	5 months	−	
169	2759	+	+		Extensive carcinoma of stump	Carcinoma unclassified			Epidermoid carcinoma, grade III
	3301	A few cells suggesting well differentiated carcinoma	+(?)	1½ months		Epidermoid carcinoma, grade III	During radiation	−	
171	2763	+	+		Negative		1 year	+	Epidermoid carcinoma
180	2940	Suggests carcinoma	+		Uncertain	Necrotic tissue(?)	2 months	−	Epidermoid carcinoma
188	3032	+	+		Negative		4 months	−	Epidermoid carcinoma
191	3035	Atypical carcinoma cells(?)	Radiation reaction(?), tumor(?)		Negative		2 years		Epidermoid carcinoma, grade III
193	3037	−	+		Carcinoma	Adenocarcinoma	4 months	−	Adenocarcinoma
197	3141	3 cells very suggestive of carcinoma	?		Negative		3 years		Epidermoid carcinoma, ungraded

TABLE VI—*continued*

Case no.	Smear no.	First diagnosis	Final diagnosis	Time between smears	Clinical data: Simultaneous clinical diagnosis	Clinical data: Simultaneous biopsy diagnosis	Clinical data: Time between radiation and smears	Radiation reaction	Type of carcinoma
200	3145	Adenocarcinoma(?)	+		Carcinoma	Carcinoma with radiation reaction	During radiation	−	Epidermoid carcinoma
	3229	+	+	5 days		Carcinoma with radiation reaction	During radiation	−	
209	3203	−(?)	−(?)		Carcinoma		7 months	+	Epidermoid carcinoma, grade I
213	3207	+	+		Recurrent carcinoma(?)	No evidence of tumor	2 years	−	Not specified
228	3349	Cells poorly preserved, suggesting carcinoma	+(?)		Radiation reaction	−	10½ months	−	Papillary adenocarcinoma of ovary
235	3531	+	+		No tumor		10 months		Epidermoid carcinoma, grade III

There are four cases in Tables IV, V, and VI in which a positive diagnosis on the smear was the first evidence of carcinoma: a case of carcinoma *in situ;* 3 cases of "hidden" or unsuspected carcinoma, including an adenocarcinoma and one post-radiation recurrence. More weighty evidence than our 4 cases has been given by others with regard to "hidden carcinoma" and carcinoma *in situ.* Papanicolaou and Traut [2] reported that among the 193 cases of malignant tumors of the genital tract diagnosed by the vaginal smear, 9 were unsuspected clinically. Meigs *et al.*[3] reported 3 cases possibly of this sort but still unproved when reported. Carcinoma *in situ* was reported by Papanicolaou and Traut in 7 of 127 carcinomas of the cervix, 100 being squamous-celled carcinoma. This is an exceptionally high proportion of cases. Meigs *et al.* reported 10 (22 per cent) clinically early carcinomas of the cervix and 5 early cases of carcinoma of the endometrium. The diagnosis was confirmed in each of these 15 cases.* The only early carcinoma in our series, excepting the carcinoma *in situ* (case 31, Table V), was an unexpected finding after hysterectomy (case 18, Table V). This was a completely keratinized, well differentiated, slightly invasive carcinoma. It did not produce cells in the smear recognizable as malignant.

We wish to emphasize a third condition—recurrence after radiation—in which the smear may be invaluable in demonstrating carcinoma. In such cases there may be a real hesitancy in performing a biopsy. In our material there are 13 cases of recurrent carcinoma proved by biopsy 1 to 4 years after radiation. The recurrences under 1 year are not considered in this connection because of the difficulty of distinguishing between persistence, radiation necrosis of carcinoma, and recurrence. In only 1 of these 13 cases did the smear give the first indication of recurrence. Early recurrent carcinoma may be very difficult to recognize due to radiation reaction.

Radiation reaction as seen in sections of tissue is recognized not by a single specific change but by a concurrence of changes in stroma, blood vessels, and epithelial cells. Anaplasia, often prominent in radiation reaction, is readily distinguished from neoplasia in sections but not so in smears. The isolated radiated cells may closely resemble malignant cells. There are two prominent features of smears showing radiation reaction: a high proportion of "basal" cells and variation in their appearance. These "basal" cells are large with swollen nuclei, which tend to be pale rather than hyperchromatic or very small with

* Jones *et al.* reported three unsuspected instances in patients attending an endocrine clinic. (Jones, C. A., Neustaedter, T., and Mackenzie, L. L. The value of vaginal smears in the diagnosis of early malignancy. *Am. J. Obst. & Gynec.*, 1945, 49, 159–168.)

deeply pyknotic nuclei. There is usually variation in shape as well as in size. Cells of undetermined type may represent injured endocervical epithelium (Fig. 3). Obvious signs of degeneration are seen in some of the cells as, for example, the heavily spotted nuclei, with large distinct clumps of chromatin sometimes without a nuclear membrane (Fig. 5). Such nuclei may be seen in cervicitis and are identical with nuclei in some early epidermoid carcinomas (Figs. 33 and 39 of Plate E [2]), and others resemble Figures 11 and 27 in that plate. Many of the radiated cells closely resemble the carcinoma cells of a grade I epidermoid carcinoma depicted in Figure 6, Plate J.[2] Some of these very atypical cells are seen in patients who have remained without evidence of disease 5 or more years after radiation treatment. This similarity of the radiated and malignant cell is well brought out by our Figures 3 and 4, which represent radiated epithelium of endocervical glands. The preparation was made from a uterus removed after radiation for epidermoid carcinoma, grade I, of the cervix. There was no residual tumor found in multiple sections but there was marked radiation reaction of the epithelium of the endocervix.

Some of the cell changes prominent in radiation reaction may also be seen in nonirradiated elderly patients, more often in the seventh and eighth decades than earlier, and usually are associated with infection. The differentiation of radiation reaction from changes due to atrophy and infection on the one hand and from malignant cells on the other depends on the cell picture as a whole rather than on the appearance of certain cell types. Thus, in radiation reaction there are more cells that differ from one another in appearance and usually more anaplastic cells than are seen in nonradiated benign conditions. We have reviewed all of our smears in the series without reference to other data, for the purpose of determining the presence of radiation reaction. The extent of radiation reaction is unpredictable and cannot be correlated with the time element or the amount of radiation. Only 46 of 157 smears from radiated patients showed definite radiation reaction. Twenty-three others were suggestive of radiation reaction. There were 10 smears which we thought showed radiation reaction but the patients had not been radiated. Four of the patients were over 70 years; 4 were between 60 and 70; 2 were 47 and 56 years old. In 3 of the cases carcinoma was present in the smears; the others showed cervicitis.

We cannot show as clear-cut results with the smears as others have reported. We have found "accuracy" a thing not easily determined as applied to diagnosis of smears. The fact that the diagnosis confirms other data does not necessarily attest to its correctness. Thus in a case reported by Meigs *et al.*, in which the vaginal smear was positive, the

initial pathologic diagnosis on the removed uterus was negative for tumor, but multiple sections demonstrated a minute focus of tumor in 2 of 50 slides.[3]

Our report leaves unanswered the all-important question in respect to the consistency with which the smear represents the condition of the genital tract. It is the general impression of those using the method that if carcinoma is present the cells may be found fairly consistently in the smears. Meigs *et al.* made the statement that of 16 successive smears taken from a woman known to have cancer, in a period of 7 days, only 6 smears contained cancer cells.[3] This statement alone would count heavily against the practicability of the method. It does demonstrate that the diagnosis of cases by multiple smears is more accurate than diagnosis based on single smears. The problem of accuracy of interpretation of the smear and the problem of the representative character of the smear have each to be considered. Since thus far no data have been presented on the accuracy and consistency of each smear taken on a given case, we have given in detail our interpretation of each smear submitted, based on pathologic criteria alone, and have correlated this with other data of the case. The high proportion of radiated cases in this group and the limited experience afforded by the small sample permit only limited conclusions as to the general usefulness of the method.

Summary and Conclusions

A total of 341 vaginal smears from 233 women were studied to determine the place of this method in routine diagnosis of the presence or absence of uterine carcinoma.

A mistaken positive diagnosis of a negative smear should be a problem only in exceptional cases of radiation reaction or of senile atrophy with infection.

False negative diagnoses are more difficult to avoid. There are certain types of carcinoma cells which are difficult to recognize without a good deal of experience.

Interpretation of smears made after radiation treatment demands more experience than other smears because of the changes in epithelium induced by radiation.

The smear may be valuable in diagnosis to detect recurrence after radiation since in these cases biopsy is preferably avoided.

As a subsidiary test, the vaginal smear may be especially useful in cases of "hidden carcinoma."

A pathologist will not need special experience to recognize untreated carcinoma of the cervix of low to moderate malignancy, and a relatively

short period of training may suffice for a technician. On the other hand, smears from carcinoma of the cervix of high malignancy, sloughing tumors, some adenocarcinomas of the endometrium and endocervix, as well as irradiated carcinomas may be difficult problems for a pathologist even after some familiarity with the method.

The time taken in examination of the smear, always provided it is carefully prepared, may vary from 2 or 3 to 20 minutes. This limit is set from the standpoint of laboratory practice, not research. This method as a means of final diagnosis has yet to be clearly established. Before this is possible, there must be more specific information upon the limitations and advantages of the method based on larger series of cases. It is our impression that this procedure is promising in a high degree.

As a screening test for detecting the existence of cervical or endometrial cancer in large groups of women it may well be of value.

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DESCRIPTION OF PLATES

Plate 96

Fig. 1. Malignant cell in vaginal smear from patient with epidermoid carcinoma, grade III, of cervix. × 2000.

Fig. 2. Malignant cell in vaginal smear from patient with carcinoma *in situ*. × 2000.

Figs. 3 and 4. Endocervical epithelium showing radiation reaction. This is photographed from a section of the removed uterus. × 2000.

Fig. 5. Cell showing nuclear degeneration from vaginal smear of patient radiated for carcinoma. × 4000.

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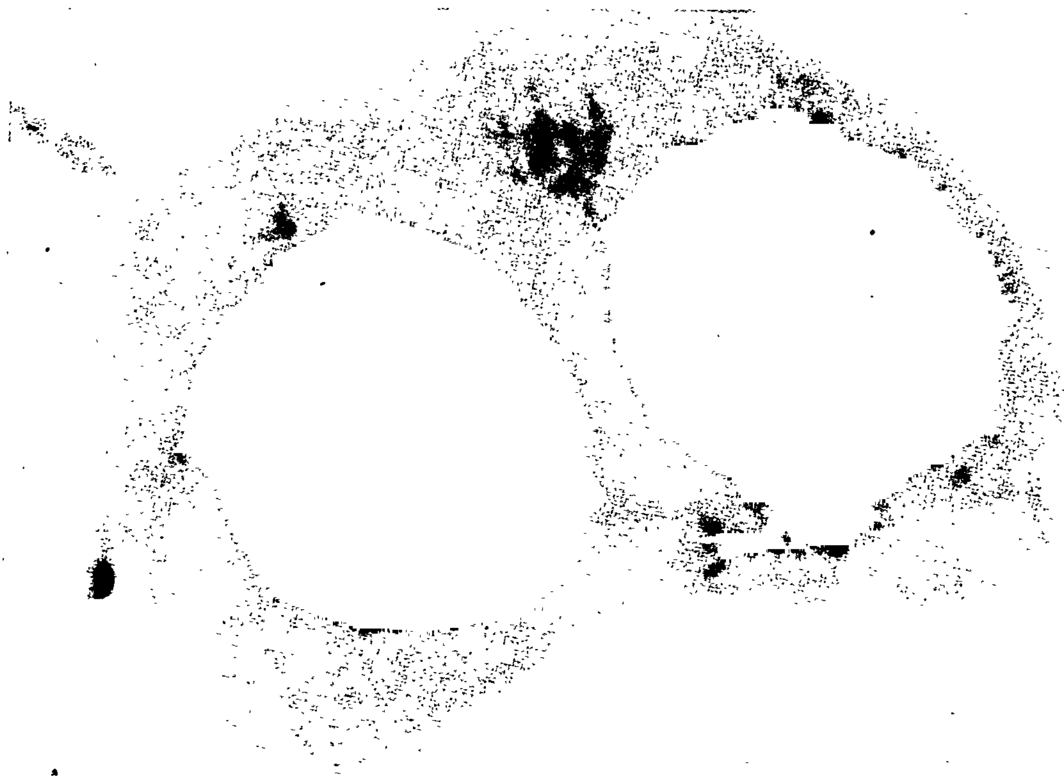
Plate 97

Fig. 6. Two cells from smear of patient with carcinoma *in situ*. × 4000.

Fig. 7. Atypical endocervical epithelium showing results of radiation. Photograph of cells in section from removed uterus. × 4000.

Fig. 8. Endocervical epithelium from same case as Figure 7. × 2000.



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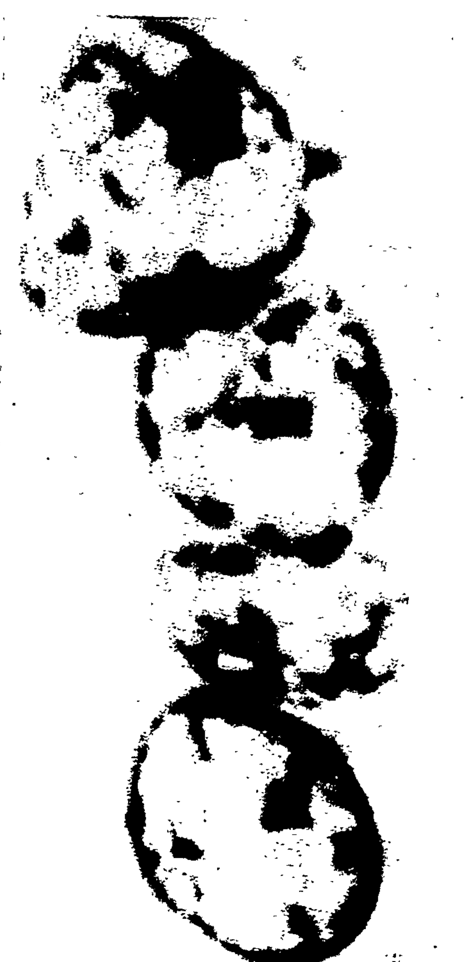

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PLATE 98

These photographs are of cells in smears. × 2000.

FIG. 9. Epidermoid carcinoma, grade III.

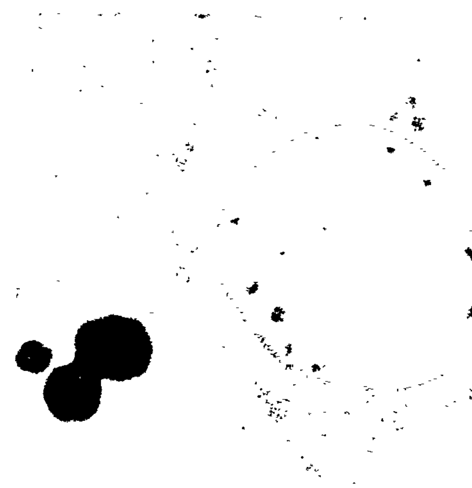
FIG. 10. Epidermoid carcinoma, ungraded.

FIG. 11. Adenocarcinoma.

FIG. 12. Atypical basal cell.

FIG. 13. Adenocarcinoma.

9.



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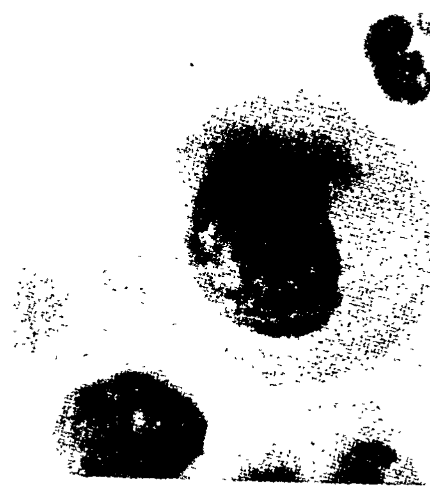

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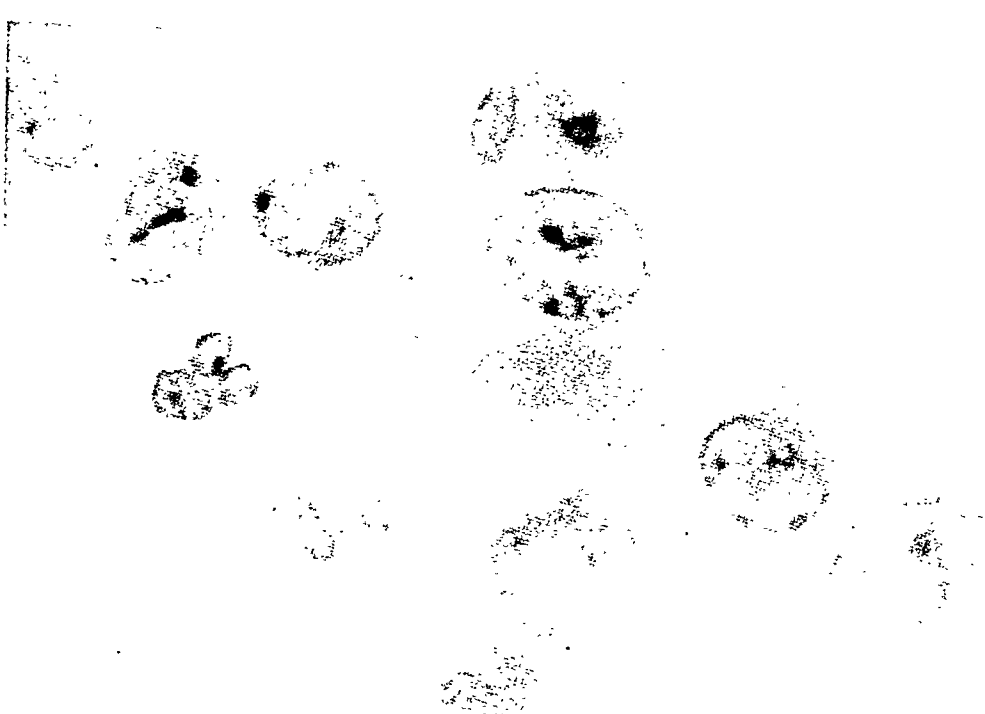

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Vaginal Smear in Diagnosis

A COMPARATIVE STUDY OF THE PATHOLOGY OF SCRUB TYPHUS (TSUTSUGAMUSHI DISEASE) AND OTHER RICKETTSIAL DISEASES *

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During and immediately after the last war, literally millions of lives of both the civilian and military population of the Near East were sacrificed to the louse-borne typhus fever. In this war, although our troops have been exposed to endemic and epidemic foci, not a single American soldier has died of louse-borne typhus fever. This record, brought about in large part through the use of a consistently effective vaccine, surely constitutes one of the outstanding achievements in preventive medicine. Unfortunately, the situation with regard to scrub typhus is considerably different. To date, no reliable vaccine, antiserum, or chemotherapeutic agent has been devised to combat the disease which is precariously widespread throughout the Far East. It is therefore imperative that the facts as offered by all the pertinent sciences, including pathology, be collected and integrated into a unified workable account. Toward this end, there have been collected at the Army Institute of Pathology, through the efforts of the U. S. A. Typhus Commission † and of individual medical officers, 100 cases of scrub typhus of which the first 78 form the basis of this report, 24 cases of epidemic louse-borne typhus, and 12 cases of Rocky Mountain spotted fever.‡ In addition, through the kindness of Dr. R. D. Lillie, Senior Surgeon of the U.S. Public Health Service, the histologic slides of 2 cases of American "Q" fever were made available. It was our purpose not only to try to learn the lesions of scrub typhus, but to determine if there were any changes which were sufficiently characteristic and constant as to permit a histologic differentiation of the various typhus fevers.

NOSOLOGY

For purposes of practical nosologic orientation we have tabulated the rickettsioses as shown in Table I.

In this communication we are concerned primarily with the tsutsugamushi group which, like the spotted fever group, has been subdivided

* Received for publication, January 15, 1945.

† This material consists of pathologic specimens from 24 cases of louse-borne typhus fever observed in Egypt by Lt. Comdr. W. B. McAllister, M.C. (U.S.N.R.), member of the U.S.A. Typhus Commission. Of these cases, 23 were used as the basis for comparison in this study. Grateful acknowledgment is made to Lt. Comdr. McAllister for permission to use this material.

‡ The tissues of 72 cases of scrub typhus and of all the cases of Rocky Mountain spotted fever were fixed in formaldehyde solution; the tissues of the 6 remaining cases of scrub typhus and of all the epidemic typhus were fixed in Regaud's solution.

into various entities that were born chiefly of geographic barriers rather than real, inherent distinctions. While it is true that certain differences exist, such as great discrepancies in mortality rates, as well as the absence of a primary lesion or eschar in at least one form (Malayan),

TABLE I
Classification of the Rickettsioses

Disease	Vector	Weil-Felix Reaction †
I. Typhus Group		OX19 +++, OX2 +, OXK −
A. Epidemic typhus (tabardillo)	Louse	
Brill's disease *		
B. Murine (endemic) typhus	Flea	
II. Spotted fever group		
A. Rocky Mountain spotted fever	Tick	OX19 +, OX2 +, OXK +
B. São Paulo fever		
C. Colombian spotted fever		
D. Kenya typhus		
E. South African tick fever		
F. Fièvre boutonneuse		
III. Tsutsugamushi fever group		
A. Tsutsugamushi (Japan, Formosa, etc.)	Larval mite	OX19 −, OX2 −, OXK +++
B. Scrub typhus (New Guinea, etc.)		
C. Queensland coastal fever		
D. Malayan (rural, tropical) typhus		
E. Sumatran mite typhus		
IV. Miscellaneous group		
A. "Q" fever		OX19 −, OX2 −, OXK −
1. Australian "Q" fever	Tick (?)	
2. American "Q" fever	Tick (?)	
B. Trench fever	Louse	
C. Colorado tick fever ‡	Tick	
D. Texas tick fever ‡	Tick	

* Brill's disease is currently regarded as a recrudescence of latent louse-borne, epidemic typhus.

† The degrees of reaction as herein indicated are, of course, arbitrary. Variations may occur, particularly in the spotted fever group.

‡ Proof of the rickettsial etiology of these diseases is not yet universally accepted.

there is considerable immunologic evidence that these diseases are essentially the same.[1-3] The phenomenon of differences in reactions of different peoples to an organism, which itself may vary in habits, hardiness, and heritage, has many well known analogies in Medicine; to wit, the markedly varied response of certain nationalities and races to tuberculosis, spirochetoses, streptococcal infections, etc. The cases in the current series developed in New Guinea and the disease will hereafter be called "scrub typhus."

Clinical Picture

Detailed clinical, epidemiologic, and laboratory reports will be forthcoming from other sources. Therefore, only a brief summation of these

data will be included here. The etiologic agent is a rickettsia (*R. orientalis* or *R. tsutsugamushi*) of which the reservoir is probably the rat, field mouse, or bandicoot. The rickettsiae are transmitted to man by the larval forms of the mites, *Trombicula akamushi*, *T. deliensis*, *T. hirsti*, and others. The larval mites are encountered in the tall lalang or kunai grass, brush, or secondary jungle growth consisting of stunted trees or "scrub." The larval mite acquires rickettsiae by sucking a meal of blood from a vertebrate host. The organisms are transmitted through the ova to succeeding generations of mites. The adult mites do not feed on man. At the site of the bite, a characteristic primary lesion, called an eschar, develops. The host may not notice the lesion until the disease is clinically evident, or he may fail to notice it entirely. The incubation period varies from 10 to 18 days.[4] The onset is sudden and is characterized by chilliness or rigors, headache, and fever. A macular or slightly papular rash appears on the 5th to 8th day, usually fading in 4 or 5 days. The rash is to be distinguished from dengue, measles, dermatitis medicamentosa, etc. An enanthem may develop on the soft palate, but the buccal surface of the cheeks is spared. In severe cases, the rash may be petechial. The lymph nodes, especially those draining the site of the eschar, are enlarged and slightly tender. Cough occurs early and is troublesome. Pneumonia, both bronchopneumonia and the interstitial variety, is frequent. Injected conjunctivae and photophobia, as in other typhus fevers, are common. The headache and fever persist and may be accompanied by nausea, vomiting, diarrhea, epistaxis, apathy, transient deafness, stiffness of neck, clouding of sensorium, and often querulousness, insomnia, delirium, convulsions, pareses and paresthesias, and disturbances of superficial and deep reflexes. In severe cases, the pulse is rapid; hypotension, shock, and vasomotor collapse ensue; and death occurs, as a rule, from the 10th to the 17th day. In our series, 81 per cent of the deaths occurred in this period. The mortality rate ranges from 2 to 10 per cent in the New Guinea region. The rate is high in patients over 40 years of age, and in those whose health prior to the onset of scrub typhus was compromised by debilitating diseases such as malaria or dysentery. The age of the patients in the present series ranged from 18 to 40 years.

Laboratory Data

The Weil-Felix reaction, agglutination of the Kingsbury strain (OXK), tends to become positive about the tenth day and to reach its maximum near the middle of the third week of illness. In the current series, the maximum titer was 1:1280, but titers as high as 1:50,000 have been recorded. A single agglutination of 1:160 is regarded arbi-

trarily as diagnostic although a lower initial titer may be considered significant if there is a subsequent rise. A negative Weil-Felix reaction does not preclude the diagnosis of scrub typhus inasmuch as rickettsiae have been recovered in such cases by the inoculation of blood into white mice. There is no parallel between the height of the titer of the Weil-Felix reaction and the severity of the disease. Anemia and leukopenia are usually present, particularly early in the disease. The differential count is inclined to be normal but may show a relative lymphocytosis. In severe cases the urine usually contains albumin (2 plus to 4 plus); granular casts are common. The data available to us on the chemical state of the blood and on the cerebrospinal fluid are insufficient to serve as a basis for conclusions.

Observations

SKIN

Eschar

The lesion produced by the bite of the mite infected with rickettsiae produces in rapid order a papule, vesico-papule, excoriated papule, frank eschar, ulcer, and finally a small scar. This evolution of the lesion takes place in about 3 to 4 weeks, but may be prolonged if modified by secondary infection such as is prone to occur in the groin or axilla. The maximum diameter of the eschar averages approximately 5 mm. and is surrounded by an erythematous areola of about the same diameter. An eschar was recorded as having been present in 94 per cent of the cases. In 4 of our cases, the eschar was removed during life without any noticeable effect on the course or pathologic features of the disease. In 2 cases, multiple (2) eschars were observed.

Eleven eschars from this series were available for histologic study. In addition, 9 eschars from other cases of scrub typhus were examined. Sections of other stages of the lesion were not secured.

The distribution of the eschars was as follows:

Location	*Cases*
Genitals	11
Axilla	11
Thigh	9
Abdomen	8
Knee and popliteal space	7
Chest	5
Groin	5
Miscellaneous	16
(Back, 3; neck, 3; ankle, 3; buttock, 3; arm, 2; lower leg, 1; heel, 1)	

Histology of Eschar

The eschar appeared to begin as a small intra-epidermal pressure vesicle. The vesicle then became purulent and advanced aggressively in all directions. The contents included serum, intact as well as karyorrhectic polymorphonuclear leukocytes mixed with various mononuclear cells, fragments of keratin, blood, and colonies of bacteria, principally staphylococci (Fig. 1). At this stage, the histologic picture simulated that of hydroa aestivale, a crusting vesicular eruption related to exposure to sunlight. The roof of the vesicle, which was constantly parakeratotic in contrast to the nonnucleated stratum corneum of the nearby epidermis, was disrupted in all of the specimens. In extending inward toward the corium, the pustule tended to destroy the base originally formed by the spinous layer of epidermis, so that after a week or so the dermis formed the floor of the ulcer and the lesion could no longer be recognizable as primarily intra-epidermal. However, even in such advanced lesions, a clue to the morphogenesis of the lesion was found occasionally at the angles of the eschar, where the vesicle might still be seen intra-epidermally, usually with floating wisps of keratin as the remnants of the roof (Figs. 3 and 4).

The epidermis adjacent to the ulcer was usually slightly to moderately acanthotic, with intracellular edema of the rete malpighii and a tendency toward hyperchromatism, some loss of polarity, and crowding of the cells of the basal layer (Figs. 4 and 5). The edema of the papillary layer adjacent to the necrotic tissue was usually slight but in one instance was so marked as to have caused vesiculation.

The suppuration and necrosis extended through the papillary layer to about the mid-corium, as a rule (Fig. 1). In one of the cases in which the skin was from a site normally very thin, the necrosis reached the subcutaneous fat. In practically all instances the suppurative portion of the lesion, while not sharply demarcated, was distinctly zonal. Beyond this zone the infiltrate tended to be localized to the appendages and vessels, and was composed almost exclusively of various mononuclear cells (Fig. 6). The collagenous fibers at the base of the lesion showed a form of degeneration that is seen following severe, acute injury, such as may be produced by chemical, thermal, physical, or electrical agents. The fibers were swollen, acidophilic, homogeneous, and showed loss or distortion of nuclei. We have observed similar coagulative changes following other bites, for example, spider bites. There were also small hemorrhages within and immediately below the floor of degenerated collagen.

The dense collections of mononuclear cells about the coils of sweat glands, hair follicles, sebaceous glands, nerves, arteries, and veins were in striking contrast to the polymorphonuclear reaction in the upper

zone. These mononuclear cells included lymphocytes, plasma cells, many mast cells, and various forms of large macrophages (Fig. 6). Some of these histiocytes were binucleated so as to resemble the Sternberg-Reed cell, a type of cell, incidentally, which we have observed in the cutaneous reactions to tick bites. Unlike the latter reaction, eosinophilic leukocytes were practically absent in the eschar of scrub typhus (Fig. 7). In one case there was a focus of degeneration of dermal collagen containing a fragment of the mite and surrounded radially by foreign body giant cells (Fig. 8). The vessels throughout the corium, beneath and adjacent to the main site of the lesion, were dilated and hyperemic. Within the upper acute inflammatory zone, there was evidence of acute thrombophlebitis and arteritis such as would be seen in any purulent focus. However, of considerable interest were the veins which were located at a distance from the zone of suppuration and necrosis, but which, nevertheless, showed evidence of intimal damage. There were small collections of mononuclear cells—lymphocytes, plasma cells, and macrophages—which infiltrated the intima and lifted up the endothelium over them (Figs. 9 and 10). A single cross section of vein might have two or three such cellular, intimal mounds. Subendothelial vacuolization, such as may be found in association with allergic reactions, as in an asthmatic lung, was observed. In some instances, endothelial cells of veins were enlarged and hyperchromatic. Occasionally a small, nonocclusive thrombus was attached, but frequently there was a focal disintegration of the intima causing a little hillock to form (Fig. 10). This intimal cushion simulated a thrombus but appeared actually to represent an intimal verrucal swelling such as occurs in cardiac valves.[5] This type of phlebitis was found in other organs quite apart from suppurative foci and may presumptively be attributed to the direct action of rickettsiae rather than to the secondary contiguous effects of the purulent reaction of the eschar. Giemsa stains revealed bodies which we interpreted as rickettsiae in venous endothelium in only one eschar. In addition to the intimal verrucae, platelet thrombi also were seen.

Macule of Scrub Typhus

Sections of the rash were available from 9 cases of scrub typhus. These sections were secured at autopsy of patients of whom 7 were ill from 11 to 14 days; of the 2 remaining, 1 was ill 2 days, and the other survived 26 days. With the exception of the patient whose illness lasted 2 days, the histologic picture was essentially similar in all cases. The epidermis was not remarkable. There was moderate edema of the papillary layer with marked hyperemia of the upper layer of the corium. The arterioles, capillaries, and veins were surrounded, gener-

ally eccentrically, by mononuclear cells consisting of lymphocytes, plasma cells, numerous mast cells, and various macrophages. These collections constituted the equivalent of the "typhus nodule" (Figs. 11 to 13). In the one instance of the patient who died after 2 days, there were included in the infiltrate about some of the vessels considerable numbers of polymorphonuclear leukocytes.

The vascular changes were confined to capillaries, venules, and veins. Small platelet thrombi were observed in the vessels in each case. Other vascular changes consisted of enlargement and hyperchromatism of one or more endothelial cells of a capillary or vein, occasionally associated with subendothelial vacuolization or edema, necrosis and swelling of the underlying intima. These changes might or might not be associated with an infiltration of several lymphocytes, plasma cells, or macrophages, and verrucal outpouching of that portion of the intima. Frequently several such hillocks were seen in the same cross section of a vein. As a rule, such phlebitic areas were focal, the intervening portions of the wall of the vein usually appearing quite normal, even within the same plane of the section. Moreover, there was no constant relationship between the reaction in the vessels and the degree of perivascular infiltration or other extra-vascular, contiguous areas of inflammation; rather, these changes appeared to constitute an expression of intrinsic vascular, usually intimal damage. Veins of the hypodermis, fascia, and underlying skeletal muscle were similarly involved.

The appendages were not remarkable except for the frequent degenerative changes in the coils of the sweat glands. These changes ranged from epithelial vacuolization to pyknosis of the nuclei and desquamation of the fragments of the lining cells (Fig. 15). However, these findings were nonspecific and may be observed in many dermatoses. Frequently there were small mononuclear foci of panniculitis, occasionally associated with atrophy of the included fat.

Macule of Louse-Borne Typhus

The macule of epidemic typhus was histologically essentially similar to that of scrub typhus, although several differences were found. In the first place, although capillary thrombi were present in all of the available sections of macules of scrub typhus as against only 15, or 65 per cent, of the macules in epidemic typhus, the thrombi were considerably more conspicuous in the latter disease. They were more prominent not only because more thrombi occurred in a single section, but because they were larger and were associated with more pronounced endothelial changes. In epidemic typhus, the affected endothelial cells tended to be larger, more hyperchromatic, and more often disintegrated into chromatin dust, the last being a feature observed also in the capillaries of

other organs, including the glomerular capillaries. Secondly, there was a tendency to the occurrence in epidemic typhus of a necrotizing arteritis and thrombo-arteritis, not found in scrub typhus. The necrosis might extend through the entire wall of the artery, unlike the lesser degree of involvement that is stated to occur in the experimental animal infected with the rickettsiae of epidemic typhus.[6] Indeed, in the human skin of cases of epidemic typhus there may be infarct-like hemorrhagic suppurative necrosis of the portions of corium in association with severe arteritis. Thirdly, whereas no significant changes were noted in the epidermis of the macule of scrub typhus, minor changes consisting of focal spongiosis, patchy parakeratosis and focal "liquefaction degeneration" of the basal layers were infrequently observed in the skin of patients with louse-borne typhus.

Macule of Rocky Mountain Spotted Fever

The changes in the skin of patients with Rocky Mountain spotted fever approached more closely those of epidemic typhus than those of scrub typhus. However, the scrotal changes were generally considerably more advanced. There was a marked tendency for scrotal hemorrhage and actual gangrene to occur. The basis for the necrosis was found in extensive, necrotic panarteritis and thrombo-arteritis of the corium. Frequently the necrotic vessels were surrounded by an exudate in which the polymorphonuclear leukocyte predominated. These gangrenous areas were superficially reminiscent, from a histologic point of view, of the primary eschar of scrub typhus, but there were several important differences, especially the absence of thrombo-arteritis at a distance from the gangrenous area of the eschar. In less markedly involved arteries in spotted fever, the walls were focally infiltrated with mononuclear cells and polymorphonuclear leukocytes, often associated with fibrinoid necrosis of part of the wall and much karyorrhexis of the indigenous as well as the infiltrating cells of the vessels.

In addition to the macules of Rocky Mountain spotted fever, there were available sections of macules from 17 cases of fièvre boutonneuse. As might be expected, these were quite similar to those of Rocky Mountain spotted fever except, perhaps, for the greater edema of the papillary layer and the more frequent foci of acute panarteritis in fièvre boutonneuse. No sections of the primary lesion (tache noire) were studied.

HEART

Myocardium

Sections from 74 hearts were examined and in most instances multiple sections were available. Of these, only 5, or 7 per cent, were con-

sidered essentially negative. In the remainder, various degrees of interstitial myocarditis were present. The cells of the infiltrate consisted of small and large lymphocytes, plasma cells, several kinds of mononuclear phagocytes, perhaps in different stages of development, Anitschkow myocytes, scattered mast cells, and infrequently neutrophilic and eosinophilic leukocytes. The character of the infiltrate resembled that in other organs, except that the myocyte was confined to the heart. "Giant cells" were formed by binucleate plasma cells or macrophages. Occasionally the macrophages showed evidence of erythrophagocytosis and cytophagocytosis. The type of infiltrate was similar in the three varieties of typhus, although polymorphonuclear leukocytes were prone to occur more often in spotted fever. The infiltrate was located principally between muscle fibers, although it was

TABLE II

Comparative Degree of Interstitial Myocarditis

Disease	0		+		++		+++		++++		Total cases
	Cases	%	Cases	%	Cases	%	Cases	%	Cases	%	
Scrub typhus	5	7	32	44	27	35	8	11	2	3	74
Epidemic typhus	4	17	12	50	5	21	3	12	0	0	24
R. M. s. f.	2	17	7	58	3	25	0	0	0	0	12

frequently found also in the periarterial fibrous tissue and rarely within the sarcoplasm of a muscle fiber (Fig. 16). Usually the infiltrate was associated with an interstitial edema recognizable by the loose disposition of the fibers and the presence of fine granular protein precipitate in the interstitium. The infiltrate varied in quantity from about one focus of several cells in each low-power field to a striking concentration in which the cells occupied approximately a third of the field. The degree of infiltration was graded 1 plus to 4 plus on the basis of these approximate limits and compared with the corresponding changes in the hearts from cases of epidemic typhus and Rocky Mountain spotted fever. The criteria for the different grades of infiltration are obviously not precise but, nevertheless, Table II does provide an index of the relative degree of myocarditis in these three conditions.

Although 12 cases of the tick-borne disease constitute a small series, it appears from this table that the myocardium in both scrub and epidemic typhus tends to suffer greater involvement than it does in spotted fever. Similarly, 49 per cent of the cases of scrub typhus have 2 plus or greater degrees of infiltration, whereas only 33 per cent of the epidemic type are in the same category. It seems, therefore, that the degree of myocarditis, in descending order of involvement, is: (1) scrub typhus; (2) epidemic typhus; (3) spotted fever.

Localization of Myocarditis. In five instances, sections from both ventricles were available. There was no evidence to support the impression that the right heart was involved more frequently than the left, that the ventricles were more severely damaged than the auricles, or that one part of the wall of the ventricle was selectively involved. However, it is true that in a given case there usually was an unequal distribution of infiltrate. This obtained not only in sections taken from different locations, but even within a single section the infiltrate might be collected in one part of the section and spare a considerable portion of the remainder. Because of this uneven distribution of myocarditis, an erroneous impression of a selective localization of infiltrate might be acquired from the examination of an insufficient number of slides. Moreover, just as the quantity of infiltrate varies, so may its character. That is to say, in one section, the plasma cell may be the predominant element; in another, the acidophilic macrophage may be most numerous; and in still another section from the same case, the Anitschkow myocyte may predominate.

Integrity of Myocardial Fibers. Notwithstanding the abundance of infiltrate, it is our impression that, with one qualification, there is a remarkable preservation of myocardial fibers in all three types of typhus. Exceptionally, there was found an isolated, swollen, partially hyalinized fiber, in the sarcoplasm of which there were one or two karyorrhectic inflammatory cells; or, rarely, a group of fibers interspersed with mononuclear cells showed evidence suggestive of an ischemic atrophy (Fig. 20). However, the impression of the morphologic integrity of the fibers must be qualified by the occurrence of fragmentation of the fibers, particularly at the sites of inflammatory foci (Fig. 16). Although the markings of such fibers remained distinct, the fragmented edges frayed off into fine fibrils which were then lost in the inflammatory zone. Small hemorrhages were rarely observed in these areas or, indeed, elsewhere in these hearts. It is acknowledged, of course, that the use of the diagnosis "fragmentation of myocardial fibers" is much overdone because the change is generally post-mortem or at most agonal. Nevertheless, we should be hesitant to dismiss summarily this particular form of fragmentation because of its association with foci of infiltrating cells.

Myocardial Vessels. Although the character of the infiltrate and the changes in the myocardial fibers are essentially similar in scrub typhus, epidemic typhus, and spotted fever, there are distinct differences in the involvement of the cardiac blood vessels. In the hearts of scrub typhus no artery was found showing the obvious fibrinoid degeneration that may be seen occasionally in the other types of typhus. In only one instance was an intramyocardial artery found infiltrated with mononuclear cells, localized almost exclusively to the intima. Similar subendo-

thelial foci were found in several of the main coronary arteries and in the aorta. This form of infiltration can be found in other infectious diseases. A small nonocclusive thrombus was present in one of the coronary arteries. Occasionally the endothelium of capillaries, arterioles, and arteries might be swollen and lifted off the intima by subendothelial edema. In two instances there was observed focal thrombophlebitis of small myocardial veins. Occasionally the arterioles manifested a focal, verrucal, acellular, intimal swelling which, again, was of the type seen in a variety of infectious states. Such completely nonspecific intimal swellings are associated with thrombi or so-called "granular plugs," [5] thus complicating the use of one of the principal diagnostic features of the typhus diseases.

Whereas no instance of necrotizing arteritis was found in the myocardium of scrub typhus, in 4, or 17 per cent, of the cases of epidemic typhus such examples were found. The arteritis involved the small vessels and varied in degree from focal inflammation of the intima to a marked necrosis extending not only through the entire wall but to the surrounding periarterial collagenous mantle (Fig. 22). The walls of such vessels were infiltrated with various mononuclear cells, some of which were markedly distorted or actually karyorrhectic. In places, the necrosis was of such severity as to have destroyed even traces of the normal cells of the vascular wall. This type of lesion closely resembled the arteritis seen in some of the diffuse vascular diseases such as disseminated lupus erythematosus. Focal mononuclear phlebitis, associated usually with a nonoccluding platelet thrombus, occurred about as often as arteritis in our cases of epidemic typhus. In the cases of spotted fever, vascular lesions of the heart, exclusive of the slight to moderate swelling of capillary and arteriolar endothelium, were rare and consisted of a focal, mild, mononuclear infiltration of veins with thrombosis, very much of the order of change observed in scrub typhus.

Finally, it was noted that the intramyocardial nerves may participate in the inflammatory reaction in each of the three diseases (Fig. 21). Similar neuritis was found in other organs.

Endocardium

The involvement of the mural endocardium by the mononuclear infiltrate was often striking in scrub typhus and was observed in about one-third of the cases. The infiltrate tended to be more marked in the endocardium over the papillary muscles or columnae carneae. In its severest form, the mononuclear infiltrate of lymphocytes, plasma cells, and macrophages occupied the entire thickness of the endocardium. Occasionally the infiltrating cells were palisaded so as to simulate a rheumatic reaction. In its milder forms, it was not unlike the reaction

seen in other infectious diseases or following the use of serum.[7] In only one instance was a mural thrombus observed, although the presence of small pulmonary emboli in several other cases suggested the occurrence of mural thrombi in additional instances. In one case a small verruca of the mural endocardium was formed at the site of degeneration of the endothelium. As a rule, the endothelium remained intact over the infiltrate. No correlation generally was found between the degree of infiltration of the myocardium and the endocardium. The valves showed a similar infiltrate in the auricularis layer of the mitral (4 of 9 cases) and in the ventricularis of the aortic valve (3 of 4 cases). In one instance, the cellular infiltration of the spongiosa of the mitral valve was so extensive as to be of itself indistinguishable from rheumatic valvulitis. In no instance were Aschoff bodies found although the edema of the interstitium and proliferation of histiocytes and Anitschkow myocytes might superficially simulate an Aschoff body. In 2 cases, the interstitial mononuclear reaction was associated with fibrinoid degeneration of the collagen so as to suggest the interstitial myocarditis of disseminated lupus erythematosus even more strongly than that of rheumatic fever.

The endocardial infiltrate in the cases of *epidemic typhus* was of similar quality but far less intense. The endocardium showed no significant reaction in our series of cases of *spotted fever*.

Epicardium

In the epicardium were found focal perivascular infiltrations of mononuclear cells of the same type as in the myocardium. Frequently, there was a preponderance of eosinophilic macrophages, as in other infectious diseases and, incidentally, as in cases of lupus erythematosus. These cells infiltrated fat about other organs as well, *e.g.*, the perirenal fat. In several instances there was focal serous atrophy of the fat. Mononuclear cell infiltration of an epicardial nerve occurred occasionally. In only a single case was intrinsic vascular involvement seen and then merely in the form of a focal thrombophlebitis. The epicardial lesions of *epidemic typhus* and *Rocky Mountain spotted fever* are basically similar to those of scrub typhus. In one case of epidemic typhus there was observed a striking fibrinoid degeneration of a small coronary artery and adjacent fat (Fig. 25).

AORTA

The aorta frequently showed small subendothelial collections of lymphocytes, plasma cells, and macrophages. Similar cells were located about the vasa vasorum in the adventitia and outer media, often

with slight fragmentation of the elastic fibers in the vicinity. These lesions only remotely resembled the mesaortitis of syphilis. Similar lesions were seen in the aorta in cases of *epidemic typhus* and *spotted fever*.

THE LUNGS IN SCRUB TYPHUS

Interstitial pneumonitis of various degrees of intensity was found in 55 per cent of cases of scrub typhus. The earliest stage of the pneumonic reaction appeared to be a marked dilatation and hyperemia of septal capillaries followed by extensive extravasations of masses of red blood cells into the alveoli, often with an admixture of serum and a few mononuclear cells. The septa in this phase, although hyperemic, were not significantly infiltrated with inflammatory cells. The large mononuclear macrophages increased in number and lay scattered or clumped within the alveoli. These cells often contained fat and hemosiderin and might closely simulate "heart lesion" cells, especially in association with the hemorrhagic extravasations. In other areas, the alveoli might be distended merely with serous fluid. Even in these early phases there were detectable single, large, mononuclear cells with basophilic cytoplasm and hyperchromatic swollen nuclei, usually adherent to the septal wall or emerging from septal capillaries. The bronchiolar walls might be infiltrated almost exclusively with lymphocytes, plasma cells, and macrophages whereas the lumen might be filled with purulent exudate. The fasciculi of muscle of the bronchioles were often disrupted by much edema and infiltrate.

In a later stage, the septa became conspicuously thickened (two to four times) by the mononuclear cells as well as by scattered polymorphonuclear leukocytes. In small foci of atelectasis, collapsed, apposed septa might simulate actual reactive thickening of the septa. Septal capillaries were discernible but generally not hyperemic. An alveolar lining of mononuclear cells might or might not contain the mononuclear phagocytes with varying amounts of edema fluid. The alveolar fluid showed a tendency to inspissate in crescentic form adjacent to the septal wall. The interlobar septa were usually markedly edematous (Fig. 32). Purulent or nonpurulent exudate was frequently present in the bronchioles, and polymorphonuclear leukocytes might be scattered through the mononuclear infiltrate of the bronchiolar wall. In addition, there might be several alveoli or actual lobules filled with purulent exudate and representing a secondary, bacterial bronchopneumonia adjacent to a focus of interstitial pneumonitis. Commonly, areas of emphysema accompanied the pneumonitis.

At the height of the reaction, the alveoli were rimmed prominently by the mononuclear macrophages (alveolar "epithelium"). Some of

these cells showed evidence of erythrophagocytosis and cytophagocytosis; an isolated one might be in mitosis. Of considerable interest was the occasional large hyperchromatic cell which formed part of the alveolar lining (Fig. 28). This cell seemed to be identical with the lining cells observed in the interstitial pneumonitis of other diseases, including "Q" fever [8] (Fig. 36), rheumatic fever, and toxoplasmosis. Indeed, in toxoplasmosis the parasites might be found in cells of precisely this type and location (Fig. 29); similarly, rickettsiae have been observed in cells lining the alveoli of experimental animals.[9] The septa were thickened not only by the mononuclear cells, particularly macrophages, but also by edema. The macrophages within the edematous septal walls frequently elongated, and assumed the form and apparently the behavior of fibroblasts, inasmuch as such septa became collagenized. A similar, more easily recognizable process occurred in the serum-logged alveoli. In this nutrient medium, macrophages appeared to become fibroblasts and to organize the alveolar contents. The septa themselves might appear relatively ischemic. Scattered large megakaryocytes or megakaryocytoid cells were found occasionally almost occluding septal capillaries. Considerable interstitial proliferation without any reaction in the alveolar spaces might occur. This lack of positive correlation between septal and alveolar reaction was true especially of epidemic typhus.

In general there was a degree of acute, necrotizing bronchiolitis which corresponded in extent to the interstitial pneumonitis. The lumina contained much purulent exudate usually mixed with mucus, macrophages with or without fat and hemosiderin, and desquamated epithelium. The bronchiolar epithelium was frequently partially eroded. With the Brown and Brenn stain, gram-positive cocci, in pairs and short chains, were almost constantly present in the purulent exudate of the bronchioles but absent elsewhere in areas of interstitial pneumonitis. No inclusion bodies were found. The bronchiolar wall and the adjacent radiating interstitial tissue were infiltrated with lymphocytes, plasma cells, macrophages, often with scattered polymorphonuclear leukocytes and a few red blood cells.

The vascular changes in the lung were slight and consisted of infrequent platelet thrombi in the septal capillaries, mononuclear cell infiltration about arteries, arterioles, and veins and occasionally an actual phlebitis of the type seen especially in the kidney and testis. Such veins were infiltrated by various mononuclear cells which often collected as intimal mounds projecting into the lumen, at times associated with nonocclusive thrombi (Fig. 33). In 3 instances, partially organized emboli were found in the small pulmonary arteries. The

source of these emboli was probably mural endocardial thrombi, evidence of which was found in 1 case. In 2 other cases pulmonary infarcts were present, no embolus being found in the sections examined. The changes in the arteries were minimal. Occasionally there were found intimal edema and subendothelial vacuolization of the type seen in association with presumed allergic reactions as in asthmatic lungs (Fig. 81). In only one section of artery was a small thrombus observed; in this case no underlying arteritis or degeneration was apparent. In no instance was the histologic picture of periarteritis nodosa found.

The *pleura* was usually not significantly altered. In several cases, a fibrinous or fibrinopurulent pleuritis was present, but, with one exception, this was associated with an underlying bronchopneumonia. In the pleura of 3 of the cases of interstitial pneumonitis there was observed a distinctive mononuclear reaction characterized by a single row or palisaded rows of cells that resembled the mesothelial cells of the pleura. In 2 of these instances, the original mesothelial lining was intact; in the third, it was overlaid with fibrin.

The degree of interstitial pneumonia was graded 1 plus to 4 plus and compared with the severity of the interstitial myocarditis and encephalitis. The myocarditis closely paralleled the pneumonitis, but the latter showed no correlation with the encephalitis.

TABLE III

Comparative Degree of Interstitial Pneumonitis

Disease	Degree of interstitial pneumonitis								Total cases
	0		+		++		+++		
	Cases	%	Cases	%	Cases	%	Cases	%	
Scrub typhus	32	45	7	10	23	32	9	13	71
Epidemic typhus	15	65	3	13	5	22	0	0	23
R. M. s. f.	10	91	0	0	1	9	0	0	11

In addition to the interstitial pneumonitis, bacterial bronchopneumonia was found in 30 per cent of the cases of scrub typhus. Various degrees of a nondistinctive bronchopneumonia occurred in 78 per cent of the cases of *epidemic typhus* and in 20 per cent of our small series of cases of *Rocky Mountain spotted fever*. However, we noted evidence of interstitial pneumonitis not only in scrub typhus but in 8, or 35 per cent, of the cases of epidemic typhus and in 1 of the 11 cases of spotted fever (Fig. 35). In no instance did the intensity of the interstitial pneumonitis of the latter diseases even approach that found in many cases of scrub typhus. Table III indicates the relative degree of involvement in the three diseases.

LIVER

Scrub Typhus. Sections of liver from 70 cases were examined. The changes of principal interest were as follows:

1. An almost constant erythrophagocytosis and cytophagocytosis by swollen Kupffer cells.

2. An excess amount of fat, principally periportal, in 13, or 19 per cent, of the cases.

3. An increase in the sinusoidal cells. These cells include the swollen Kupffer cells, lymphocytes, plasma cells, and basophilic macrophages.

4. Foci of necrosis in a small number of cases (6 cases or 9 per cent) (Figs. 37 to 39). These foci averaged about the diameter of a pancreatic islet although in one case they were several times that size. They consisted of partially or completely lysed parenchymal cells with collapsed or dilated sinusoids. There often seemed to be a focal increase of Kupffer cells, but this impression was due merely to an apparent, rather than actual, increase of these cells following the collapse of the sinusoids and the loss of hepatic cells. In some foci there was an accompanying infiltration of lymphocytes, plasma cells, and macrophages, and occasionally polymorphonuclear leukocytes. Of interest were the small clumps of vesicular cells that seemed to be forming abortive biliary canaliculi within these foci. A few of these lesions were necrotic and composed of circumscribed eosinophilic, granular, and fibrinoid material. In one instance, the lesion resembled a typhoid nodule. In addition to the focal lesions, there was marked central congestion of sinusoids with associated necrosis of parenchymal cells in three instances. In one case there was a concomitant, extensive polymorphonuclear exudation about the central veins. This alteration is identical to that observed in shock or severe passive congestion and is attributed to anoxia of the central portion of the lobules. In addition, dissociation of hepatic cords without other alterations was frequently observed. This change of normal pattern, seen in other types of sepsis as well, is attributed to post-mortem alterations.

5. Finally, there was a slight to moderate tendency toward an increased concentration of various mononuclear cells in the periportal areas.

The changes in the livers of *epidemic typhus* and *Rocky Mountain spotted fever* were essentially similar to those of scrub typhus. However, the impression was gained of a more vigorous reaction in those diseases than in scrub typhus. That is to say, the cellularity of the portal areas tended to be greater; the foci of necrosis, while of about the same frequency, were more likely to include polymorphonuclear leukocytes and to a more conspicuous degree; and phlebitis and arteri-

tis, though by no means marked, were more apparent than in scrub typhus.

SPLEEN

Scrub Typhus. The findings in the spleen resembled closely the proliferative form of "acute infectious splenitis" or "acute splenic tumor" such as is seen in typhoid fever, for example. The follicles tended to be small for the age group concerned. Unlike the picture in some other acute infectious diseases (*e.g.*, diphtheria), the follicles were more likely to be intact than to manifest central hyalinization and necrobiotic changes. Both the Billroth cords and the sinuses were inclined to be hyperemic, although frequently they contained many large mononuclear cells. Polymorphonuclear leukocytes were relatively uncommon. The predominant cell in the sinuses and the cords was the basophilic macrophage, which in this organ, particularly, has been called endothelial phagocyte, basophilic histiocyte, and acute splenic tumor cell. This is the cell which Rich,[42] on convincing evidence, has linked with the lymphoblastic series. Erythrophagocytosis and cytophagocytosis were almost constantly present, often to a pronounced degree. Of the 68 cases from which sections were examined, 5, or 7 per cent, showed definite evidence of necrosis of the pulp, ranging from small foci to large, irregular, infarct-like areas, in most respects resembling the necrosis of the lymph nodes. Isolated thrombophlebitis of trabecular veins was found in 4 cases. No association was found between the phlebitis and the splenic necrosis. In the one smear of spleen submitted, stained by the Giemsa method of Wolbach, rickettsiae were found in cells that appeared to be macrophages (Fig. 44).

The sections of spleen of both *epidemic typhus* and *Rocky Mountain spotted fever* were quite similar to those of scrub typhus with the exception of a more manifest tendency toward relatively greater numbers of polymorphonuclear leukocytes in the pulp, especially in epidemic typhus. In one case of epidemic typhus there was marked fibrosis of Billroth cords. No malarial pigment was present. In a case of Rocky Mountain spotted fever in which sickling of red blood cells was evident in the bone marrow (white male), perifollicular collections of blood were noted in the spleen. This finding was absent in the spleens in those cases of scrub and epidemic typhus in which sickling was observed in other organs.

LYMPH NODES

Scrub Typhus. Sections of lymph nodes from 47 cases were available. The changes were those due to hyperplasia or necrosis. In all instances, there were varying degrees of hyperplasia; in 18, or 38 per

cent, of the cases, the hyperplasia was accompanied by necrosis. The hyperplastic nodes were characterized by marked distention of the sinuses, principally with acidophilic macrophages. Lymphocytes and plasma cells, as well as clumps of fibrin and red blood cells, were also often included. The cytoplasm of the acidophilic histiocytes was usually granular, or finely vacuolated ("blister" histiocytes) and usually exhibited striking erythrophagocytosis and cytophagocytosis, both in the pulp and sinuses (sinus catarrh). The necrosis of the nodes ranged from focal karyorrhexis, hemorrhage, and deposits of fibrin and edema within a germinal center or sinus, to a massive, infarct-like involvement of almost an entire lymph node. Necrosis was present not only in nodes regional to the site of the eschar, but also in those in other areas. Frequently in the necrotic nodes the endothelium of capillaries and veins was swollen and karyorrhectic, occasionally with overlying small platelet thrombi. In the more intensely involved nodes, the mononuclear reaction was prone to extend through the capsule into the adjacent areolar tissue.

Unlike those of scrub typhus, the lymph nodes of both *epidemic typhus* and *Rocky Mountain spotted fever* did not show necrosis. They were generally moderately hyperplastic. The sinuses were usually packed with acidophilic macrophages showing abundant evidence of phagocytosis. In one of our cases, the sinuses of a pulmonary lymph node were filled with purulent exudate, apparently reflecting the purulent bronchopneumonia present in this case.

PANCREAS

Scrub Typhus. Sections of pancreas were available in 45 cases. In 14, or 31 per cent, there were foci of interstitial reaction consisting of lymphocytes, plasma cells, and macrophages, and, in 1 case, of polymorphonuclear leukocytes. These foci were never striking and appeared to be merely a part of the general perivascular and interstitial reaction that characterizes this disease. In 1 case, squamous metaplasia of the pancreatic ductules was observed.

The sections of pancreas in *epidemic typhus* and *Rocky Mountain spotted fever* were quite like those of scrub typhus.

ADRENAL

Scrub Typhus. Sections of the adrenal gland were examined in 52 cases. The principal changes were: (1) focal infiltrations of lymphocytes, plasma cells, and macrophages, preponderantly in or about the medulla, but also in the cortex and areolar tissue; and (2) a practically constant finding of varying degrees of so-called "tubular" degenera-

tion,[10] principally of the fascicular cords (Fig. 49). Our clinical data were inadequate for a study of the correlation between the degree of tubular degeneration of the adrenal glands and shock. A third finding of note was the zonal distribution of abundant fat in the mid-fascicular layer in 7, or 13 per cent, of the cases. In the remainder of the cases, the fat content seemed sparse, in keeping with the usual status of the adrenal in infectious diseases. Finally, slight focal thrombophlebitis was present in 3 cases; in 1 case, the main adrenal vein and its tributaries were thrombosed, with resultant infarction of most of the gland.

The adrenal glands in *epidemic typhus* were essentially similar to those of scrub typhus, although the focal mononuclear reaction was generally more conspicuous. The one significant point of difference was the occurrence in epidemic typhus of readily apparent inflammation and degeneration of capillaries and arterioles of both the parenchymal and adventitial tissues in 4 of the 24 cases. The adrenals of the cases of *Rocky Mountain spotted fever* were basically similar to those of scrub typhus. In only 1 of the cases was evidence of thrombo-arteritis seen.

KIDNEY

Scrub Typhus. Glomeruli. Evidence of early, but definite, acute diffuse glomerulonephritis was found in 19, or 30 per cent, of 64 cases examined. In some of these kidneys, the glomeruli were moderately to markedly enlarged (Fig. 52); in the majority, they were of normal size. However, regardless of the size of the glomeruli, there were, commonly, hyperplasia, hyperchromasia, and enlargement of the endothelial cells; similar changes of a lesser degree were observed in the epithelial cells. In addition, the epithelial cells of both the parietal and visceral layers of Bowman's capsule frequently showed acidophilic swelling and granular disintegration of the cytoplasm. In a few instances, the damage to the glomerular capillaries had progressed to the stage of actual karyorrhexis of the endothelial nuclei, with the formation of isolated platelet thrombi. (This degree of damage was more common in epidemic typhus.) In sections stained with hematoxylin and eosin, the impression was gained in many instances of a marked granular or hyaline thickening of the basement membrane of the glomerular capillaries. However, in sections stained with Mallory-Heidenhain's azocarmine and Masson's trichrome stains, it was found that the apparent thickening was produced generally by a syncytium of endothelial cells. However, in a few cases there was slight to moderate, uniform, hyaline, refractile thickening without conspicuous reduplication. The major common denominator of all these cases of glomerulonephritis was the moderate to complete ischemia of the

glomerular capillaries. In some cases, practically every glomerulus in the section was so affected; in others, although most of the glomeruli were ischemic, the capillary status of the remainder ranged from partial ischemia to marked engorgement. In some individual glomeruli, the majority of the loops were significantly devoid of red blood cells, although isolated loops were distended with blood. In several cases, the glomerular loops were fused to each other (Fig. 52); only rarely was a capillary loop found adherent to Bowman's capsule. In no instance was there evidence of the formation of glomerular crescents.

The histologic picture of glomerular ischemia assumed one of two forms: (1) The capillaries were distended with protein precipitate, fibrin, or, infrequently, by platelet thrombi; (2) the lumina of the capillaries were encroached upon, and practically obliterated, by enlarged, hyperchromatic endothelial cells (Fig. 53). Commonly, the afferent arteriole was appreciably dilated, ischemic, and filled with protein precipitate. Bowman's space was often distended, at times markedly, and sometimes contained much protein precipitate, both of the granular variety and in large, agglutinated, acidophilic masses. In several instances Bowman's capsule showed fibrinoid degeneration of the type seen in the acute glomerulonephritis associated with other diseases and in the renal lesions of disseminated lupus erythematosus (Fig. 53).

In 7 cases, changes qualitatively similar to those just described were confined to scattered glomeruli. These kidneys were diagnosed as acute focal glomerulonephritis.

Tubules. In practically all of the cases, there was swelling of the epithelium of the proximal convoluted tubules by cytoplasmic granules; or, in the more advanced changes, by hydropic vacuoles. These vacuoles, unlike those due to fat, appeared first at the luminar rather than basal aspect of the cell, and thence might extend to the entire cell. The vacuolization was often associated with collections of large, acidophilic hyaline droplets, located also near the lumen of the tubule rather than near the bases of the cells. These colloid droplets were morphologically identical with those seen commonly not only in acute glomerulonephritis, but also in lipoid nephrosis and following the administration of hypertonic sucrose.[11] Fat stains revealed only a few small droplets at the bases of the epithelial cells; the vacuoles alluded to above did not contain fat. No correlation was found between the degree of vacuolization and the presence of glomerulonephritis. The lumen of the proximal portion of the nephron was filled almost constantly with protein precipitate. Occasionally the proximal loops were appreciably dilated.

The principal change observed in the distal segment of the nephron was the presence of casts of hemoglobin in 44 cases, or 69 per cent. One or two such casts were usually found in the distal loops, appearing as satellites to the glomeruli, so to speak. In most cases, several nephrons in a section were so involved and were often associated with iron pigment in the tubular epithelium.[12] In a few cases only an occasional nephron failed to show such a cast. No correlation was found between the presence of these casts and glomerulonephritis or interstitial nephritis. In no case was there any indication that hemoglobin casts, *in this particular location*, were responsible for tubular blockage, epithelial damage, or renal insufficiency. However, in 2 cases, there was an obvious hemoglobinuric nephrosis characterized by numerous casts of hemoglobin in the *collecting tubules* as well as in the distal convoluted tubules, degeneration and regeneration of this portion of the nephron, dense acidophilic casts adjacent to necrotic distal tubules, phlebitis, and focal interstitial nephritis. In neither of these cases was there a record of the administration of a sulfonamide drug or of transfusions. On the other hand, in both of the cases there was an acute diffuse glomerulonephritis; and, indeed, in one of them, a conspicuous degeneration of the epithelium of the proximal convoluted tubules. In 3 other cases of acute diffuse glomerulonephritis, there was appreciable degeneration of the epithelium of the distal tubules *without* an associated hemoglobinuric nephrosis. Finally, in 4 cases, several small, amorphous, deeply basophilic, as well as crystalline spherules, each about the size of a monocyte, were found in the lumen of the distal convoluted tubules. These bodies were suggestive of crystals of calcium oxalate. In none of these 4 cases was there a history of sulfonamide administration; in 2, acute diffuse glomerulonephritis was present; in the other 2 cases there was merely focal interstitial nephritis.

Interstitium. Foci of interstitial nephritis of varying degrees constituted the most constant alteration of the kidney of scrub typhus. The infiltrate was most prone to occur at the cortico-medullary junction but might occur anywhere from the pelvis to the capsule. In the kidney, as in the other organs, the infiltrate was almost entirely mononuclear, consisting of lymphocytes, plasma cells, and basophilic histiocytes; occasionally a few neutrophilic and eosinophilic leukocytes were included. The infiltrate appeared to select particularly the walls of the veins and the perivenous tissue, as in other organs. In the cortex, especially, the interstitial infiltrate was often associated with considerable edema (Fig. 60). In 1 case, the infiltrate and edema were uniform and diffuse so that the diagnosis of acute interstitial nephritis was

warranted, although the association with a concomitant acute diffuse glomerulonephritis bespeaks a pathogenetic relationship.

Vessels. If the testis is excepted, vascular changes in scrub typhus were most common in the kidney. The changes assumed one of the following forms:

1. Swelling, hyperchromasia, hyperplasia, and rarely karyorrhexis of the endothelial cells of glomerular capillaries.
2. Thrombophlebitis, especially of the venulae rectae and interlobar veins, usually in association with adjacent interstitial mononuclear infiltrate which had extended into the wall and puffed out the intima into a focal hillock (Fig. 61). Other veins showed a local intimal vacuolization, fibrinoid degeneration, and infiltration of a few lymphocytes, plasma cells, or macrophages, unattended by periphlebitis. The endothelium might be either intact or degenerated; in the latter case, a small platelet thrombus might be present.
3. Arteriolonecrosis in 3 instances. Segmental necrosis of arterioles was observed in the midst of foci of mononuclear, interstitial infiltrations. In none of these cases was there glomerulonephritis or hypertension. In 1 of the cases, sulfathiazole and quinine had been administered; in the other 2, no history of chemotherapy was recorded.

In addition to the above alterations, there were two findings of note in the contents of the renal veins: (1) In 8 cases, all white soldiers, distinct evidence of sickling of red blood cells was found in the lumen of one or more of the interlobular veins and rarely in the arcuate arteries. There seemed to be no definite relation between sickling and the presence of glomerular lesions. In 4 of these cases, sickling was absent in other organs: in 2, sickling was found in the lungs; in the third, a lymph node; and in the fourth, a testis. No perifollicular lakes of blood, such as characterize sicklemia, were found in these cases. (2) The second feature of interest in the contents of the vessels was the marked concentration of mononuclear cells in the peritubular venules of the medulla. Frequently the lumina were filled with lymphocytes, plasma cells, and basophilic and acidophilic macrophages, the latter, especially, showing phagocytosis of red and white blood cells (Fig. 57). In many of these vessels polymorphonuclear leukocytes were entirely absent, although present in more or less normal numbers in the peripheral blood. The cells were not agglutinated and were not part of a stratum of a post-mortem clot. These cells corresponded exactly to those observed in the interstitial infiltrate of the kidney and other organs. However, no correlation was found between the degree of adjacent interstitial infiltrate and the concentration of these mononuclear cells in the lumina of the veins. A similar, although generally less conspicuous finding, was noted in other organs.

Epidemic Typhus. The changes in the kidneys from patients with epidemic typhus were qualitatively similar to those of scrub typhus, but considerably more pronounced. Acute diffuse glomerulonephritis was found in 18, or 78 per cent, of the cases; acute focal glomerulitis in 3, or 13 per cent, and essentially normal glomeruli in 2, or 9 per cent. The glomerular alterations were basically those of scrub typhus, differing in more marked swelling and hyperchromasia of the endothelial cells, more frequent occurrence of thrombosis of the glomerular capillaries and fragmentation of the endothelial cells. Similarly, focal interstitial infiltrations, calcific bodies, hemoglobin casts of the distal convolutions, and phlebitis, arteritis, and arteriolitis were more conspicuous in epidemic typhus. Sickling of red blood cells within renal veins was found in 8, or over one-third, of the 23 patients, all of whom were native Egyptians. In 7 of the 8 cases, acute diffuse glomerulonephritis was present; in the eighth, a focal glomerulitis was noted. Sickling was observed in various other organs in these cases although no perifollicular pooling of blood was found in the spleen. Concentration of mononuclear cells, some showing evidence of phagocytosis, was observed with approximately the same frequency and under the same conditions as in scrub typhus.

Rocky Mountain Spotted Fever. The lesions of the kidneys of patients with Rocky Mountain spotted fever were in some respects intermediate between those of scrub typhus and of epidemic typhus. Acute diffuse glomerulonephritis was found in 5, or 50 per cent, of the 10 cases; acute focal glomerulitis in 2 cases, and normal glomeruli in 3 cases. Focal interstitial nephritis, isolated hemoglobin casts in the distal convoluted tubules, concentration of mononuclear cells in the peritubular capillaries, and phlebitis were of the order seen in scrub typhus. A feature of decided divergence was the extensiveness of the necrotizing arteritis observed in the kidneys of patients with spotted fever. Acute necrotizing thrombophlebitis and thrombo-arteritis of the interlobular and arcuate vessels were noted in 3 cases; in 1 case, an infarct of the renal cortex was produced. Sickling was not found in these kidneys, although this phenomenon was manifest in a section of bone marrow from a white patient.

TESTIS

In the histologic study of the effects of generalized rickettsial infection in animals, the testis and its coverings have yielded more information than any other organ because of the selective tendency of certain strains to produce orchitis. For this reason, the morphologic changes in the human testis are of particular interest. In scrub typhus, the changes, as observed in sections of 36 cases, were found to be of no

great differential significance. They consisted of: (1) almost constant, degenerative changes—often present to a marked degree—of the epithelium of the testicular tubules (Fig. 63); (2) edema, early fibrosis, and often marked infiltration of lymphocytes, plasma cells, and various macrophages within the interstitium; (3) occasional hyperplasia of testicular interstitial cells; (4) frequent thrombophlebitis and rare arteritis—both associated with fibrinoid degeneration, edema, and mononuclear cellular infiltration of the wall (Figs. 62 and 79). Arteritis or thrombo-arteritis was present in 5, or 17 per cent, of the cases. Even though slight, this incidence is higher than that observed in other organs. Thrombophlebitis was noted in 7, or 19 per cent. The tunica vaginalis showed simply slight perivascular cuffing by lymphocytes, plasma cells, and macrophages. In the 6 cases from which sections of epididymis were available, slight thrombo-arteritis and thrombophlebitis were noted once. No hemorrhages were observed in the testis or epididymis.

Tubular atrophy, and interstitial edema and infiltration in the testes of patients with epidemic typhus are of about the same order as seen with scrub typhus. However, the point of divergence is the greater frequency and extent of the arteritis in the former. Of the 9 cases from which sections were available, arteritis or arteriolitis was observed in 4, or 44 per cent, a significant difference notwithstanding the small size of the sample. In 3 of these 4 cases, epididymis was included and all showed a similar acute arteritis. In a tenth case, epididymis alone was submitted and here, again, necrotizing arteritis was present. Among the sections of testis in which no arteritis and only a minimal degree of interstitial inflammation were noted, was one from a 10-year-old child. This observation is mentioned because of the tendency for severe involvement of the testes of children with Rocky Mountain spotted fever.[13] The 3 testes of spotted fever which were examined in our series resembled more closely those of scrub typhus than of epidemic typhus. All were from adults, however. The sections of scrotum, on the other hand, showed the characteristically marked changes of Rocky Mountain spotted fever, including hemorrhagic necrosis and acute necrotizing thrombo-arteritis, as described under "Skin."

BRAIN

Scrub Typhus. The involvement of the brain in scrub typhus takes the following forms: (1) mononuclear cell meningitis; (2) "typhus nodules"; (3) perivascular cuffing of arteries; (4) focal hemorrhages in parenchyma and meninges; and (5) degeneration of ganglion cells.

Leptomeningitis was usually present, having been found in 48, or

89 per cent, of the cases (Fig. 73). In 7 cases, the reaction was especially marked. In the 6 cases without meningitis, death occurred on the 2nd, 11th, 13th, and 14th days. In 24, or 44 per cent, meningitis occurred without parenchymal involvement. The degree of meningitis closely paralleled the intensity of periarterial reaction within the brain, but no correlation was found between the meningitis and other parenchymal lesions of the brain. The meningeal infiltrate consisted predominantly of acidophilic macrophages, with a sprinkling of lymphocytes and plasma cells.

The reaction in the parenchyma was characterized by the "typhus nodule" which was present in 18, or one-third, of the cases. The nodule was composed predominantly of oligodendroglial cells, particularly in its earliest or smallest form. In the larger nodules, a few microglial cells, lymphocytes, plasma cells, and macrophages were added (Fig. 67). Rarely, polymorphonuclear leukocytes were included. The number of cells composing the nodules averaged 15 to 20 (in a single plane of section), although the larger nodules contained as many as 40 cells. In 12 of the 18 cases, from 1 to 3 nodules were seen in a section of ordinary size; in the remaining one-third, from 6 to 10 nodules were found in a section. These lesions, as measured in serial sections, approximated 60 to 120 μ in diameter. The glial fibers within and adjacent to the nodules were often edematous or showed early demyelinization. All cases with nodules gave evidence of damage to ganglion cells in the form of satellitosis, particularly within the cortex but also in the pons and basal ganglia.

The cells of the nodule were grouped around capillaries which, however, usually could not be discerned in a single section, especially if stained only with hematoxylin and eosin. Because of the affinity of the basement membrane of capillaries for silver, serial sections of brain were so stained (Wilder stain). As a result, capillaries that were not evident in sections stained with hematoxylin and eosin were unmasked (Figs. 69 and 70). Such capillaries were found constantly, placed either centrally or eccentrically, within the nodule. Frequently the walls of the capillaries were disrupted (Fig. 69). When capillaries were seen in sections stained with hematoxylin and eosin, their walls were often found to be swollen and granular. Endothelial cells, when present, tended to be enlarged and hyperchromatic, as in other organs. The lumina were frequently empty; sometimes a single or several intact or laked blood cells were present; or the lumina were occupied by one or more mononuclear cells, possibly endothelial or hematogenous. Platelet thrombi and karyorrhexis of endothelial cells were not seen. Small, focal, parenchymal, apparently terminal, hemorrhages were ob-

served in 7, or 13 per cent, of the 54 cases. There was no indication of an association between the hemorrhages and the nodules.

The distribution of the nodules was not uniform. The cortex was involved with about one-third the frequency of the pons and medulla. Moreover, the cortical involvement included only the gray matter, the white matter having been spared entirely.

Epidemic Typhus. A comparison between the reactions in the brain and meninges in epidemic and scrub typhus is shown in Table IV. From this comparison, it appears that:

1. Although the meningitis of scrub typhus is slightly more frequent

TABLE IV

Comparative Distribution of Lesions of the Brain in Scrub Typhus and in Epidemic Typhus

	Scrub typhus			Epidemic typhus		
	Total cases	Positive	%	Total cases	Positive	%
Meningeal infiltrate	54	48	89	23	17	73
Nodules						
Cortex, gray	50	5	10	24	20	83
Cortex, white		0	0		0	0
Pons	48	16	33	21	17	81
Medulla	30	10	33	18	14	78
Basal ganglia	24	4	17	15	13	87
Cerebellum	30	3	10	14	11	79
Cord	10	2	20	15	13	87

and extensive than the qualitatively similar reaction in epidemic typhus, the involvement of the substance of the brain is considerably greater in the latter disease.

2. The distribution of lesions in the gray and white matter in the two diseases is the same: in both the white matter is spared, in contrast with Rocky Mountain spotted fever.

3. In the current series the case incidence of involvement of the cortex in epidemic typhus is much greater than in scrub typhus. The actual concentration of nodules in the pons, medulla, and basal ganglia in epidemic typhus is more pronounced than in the cortex. The pons and medulla are sites of predilection also in scrub typhus.

Several distinct histologic differences between scrub typhus and epidemic typhus are noted:

4. The nodules tend to be larger in epidemic typhus, averaging 55 to 75 cells as against 15 to 40 cells, and about 120 to 180 μ as against 60 to 120 μ.

5. There is somewhat greater cellular pleomorphism in the nodules of epidemic typhus, especially in the larger. Karyorrhexis is common

in the cells of the nodule of epidemic typhus and rare in that of scrub typhus.

6. In epidemic typhus, the capillaries of the nodules show much more obvious evidence of damage in the form of markedly enlarged endothelial cells, karyorrhexis of endothelial cells, and thrombosis of capillaries. Similar changes are found in arterioles without associated nodules.

Rocky Mountain Spotted Fever. The sections of brains of only 7 cases were studied. This number is insufficient to permit a tabulation of lesions as was done with the cases of scrub and epidemic typhus. However, certain essential differences are of sufficient constancy to be worth noting.

1. The white matter of the cortex was involved in 4 of the 7 cases; the gray matter was spared in every instance. This distribution of lesions is directly divergent from the finding of nodules in the gray and none in the white matter of the cortex of both scrub and epidemic typhus.

2. Unlike the characteristically constant type of nodule of scrub and epidemic typhus, the parenchymal lesion of the brain of Rocky Mountain spotted fever is basically a microinfarct seen in a variety of stages. Although the capillaries are damaged, the vascular involvement is essentially of an entirely different order in spotted fever as compared to the other two. Herein, presumably, lies the basis for the differences in the lesions. Frequently, fairly large parenchymal arteries were appreciably altered by fibrinoid degeneration of their walls, swelling, hyperplasia, hyperchromasia and karyorrhexis of the endothelial cells, with thrombosis, and karyorrhexis of some nuclei included within the thrombi. These changes extended from the arteries to the minute capillaries. The vessels so involved might be surrounded by a few glial cells and various hematogenous mononuclear cells, but even about the damaged capillaries such proliferations only superficially simulated the nodules of scrub and epidemic typhus. Instead, there was seen along the affected vascular arborization, usually at the level of the arteriole, one or another stage of a microinfarct (Fig. 72). The lesion might vary from swollen, acidophilic, granular, and hyaline masses of myelin to a ragged, moth-eaten, frayed area about one to three times the size of a renal glomerulus. As a rule these foci attracted glial cells, including cytoplasmic astrocytes.

If the basic parenchymal lesion of these three diseases is compared with corresponding lesions of nonrickettsial protozoan diseases, the encephalitis of Rocky Mountain spotted fever and toxoplasmosis may be considered to be of a similar histologic order. In contrast, the en-

cephalitis of epidemic typhus and of scrub typhus may be grouped with that of Chagas' disease. In addition, the malarial "granulomas" of the brain simulate the "microinfarcts" of spotted fever.

GASTROINTESTINAL TRACT

Sections of gastrointestinal tract from 19 cases were examined. These included 16 sections of stomach, 8 of small intestine, and 5 of large intestine. In 1 case there was a diffuse infiltration of the wall of the stomach and ileum with lymphocytes, plasma cells, macrophages, and many polymorphonuclear leukocytes. In the remainder the infiltration was minimal and was characterized by small foci of perivascular mononuclear cells, particularly in the muscular layers. In 2 cases, there were small foci of thrombophlebitis in the stomach and colon. The findings in the gastrointestinal tracts of the cases of epidemic typhus and Rocky Mountain spotted fever were not significantly different.

GALLBLADDER

Sections of gallbladder were studied in 3 cases. One case showed nothing of significance. In the other 2 cases there was a remarkable, acute diffuse cholecystitis characterized by dense infiltrations of lymphocytes, plasma cells, and macrophages in one instance, and a predominance of neutrophilic and eosinophilic leukocytes in the other. In the latter case there was thrombophlebitis of a small subserosal vein. The gallbladders of 3 cases of epidemic typhus and 1 case of Rocky Mountain spotted fever showed only a few minimal interstitial infiltrations of mononuclear cells.

URINARY BLADDER AND PROSTATE

The urinary bladder was studied in 10 cases and the prostate in 11 cases of scrub typhus. Usually only scattered, small, perivascular foci of mononuclear cells were found. In 3 instances the concentration of cells in the mucosa warranted the diagnosis of low-grade cystitis. Six of 9 cases of epidemic typhus presented chronic cystitis and 5 of these were infected locally with ova of *Schistosoma haematobium*. In the 4 cases in which sections of prostate were examined, nothing of significance was found. Sections of urinary bladder and prostate from cases of Rocky Mountain spotted fever were not available.

THYROID

Sections of thyroid from 4 cases of scrub typhus, 11 cases of epidemic typhus, and 2 cases of Rocky Mountain spotted fever showed nothing remarkable.

SKELETAL MUSCLE

The sections of diaphragm in 2 cases of each of the three diseases revealed slight focal myositis. No vascular changes were demonstrable. However, in the sections of tongue from 18 cases of epidemic typhus myositis was present in all but 1 case. Vascular lesions, including necrotizing thrombophlebitis, thrombo-arteritis, and capillaritis, were prominent in epidemic typhus. Sections of tongue from cases of scrub typhus and spotted fever were not available.

BONE MARROW

The sections of vertebral bone marrow from 10 cases of scrub typhus, 19 cases of epidemic typhus, and 3 cases of Rocky Mountain spotted fever were similar and revealed a hyperplastic marrow. Phagocytosis of red and white blood cells by macrophages was observed frequently.

DISCUSSION

In integrating the histologic data, several points of divergence of our findings from those previously recorded become evident, and certain problems in pathogenesis are posed.

Eschar

Occurrence. One of the curious phenomena of the tsutsugamushi group of diseases is the occurrence, at the site bitten by the mite, of an eschar in most of the entities and its absence in others (*e.g.*, in Malayan rural typhus). This discrepancy occurs notwithstanding the presumed essential identity of the organisms, of the trombiculae, and indeed, of the diseases themselves. In the explanation of this phenomenon there must reside information of a fundamental nature concerning the selective tropism of arthropods for man, the effect of their venom, and the mechanism of spread of their infectious inoculum.

It is known that if the rickettsiae of tsutsugamushi disease are inoculated intracutaneously in animals, a grossly typical eschar is produced. However, the subcutaneous or intramuscular injection of the organisms does not provoke an eschar although the generalized infection follows.[14] Similar observations have been made in experimental fièvre boutonneuse, a form of spotted fever characterized, too, by a primary lesion or *tache noire*.[15] On the basis of these findings it has been suggested, without strong conviction, that the larval mites may perhaps be introducing the organisms at different levels of skin in various countries and in various peoples.[16] However, the solution is probably not so simple a matter as the length of the proboscis of the larval mite, which, in its entirety, measures hardly the thickness of

average epidermis. It is therefore unlikely that the proboscis extends below the dermis. Granted that the disease is transmitted through the skin by the trombicula, the alternative explanations of the local lesion appear to be: (1) that the rickettsiae are introduced directly into lymphatic vessels, a manipulation difficult to conceive; or (2) that there is a natural, local, cutaneous immunity in certain groups of people to the effect of the bites of mites just as there is to the bites of other arthropods. The latter viewpoint would appear more tenable. However, these are problems that await solution.

Pathogenicity of Eschar. Inasmuch as the noninfected trombicular larva may produce a lesion qualitatively not sharply different from the infected eschar, according to Kawamura,[17] the question arises as to whether the primary lesion is produced by the venom of the mite or by the rickettsiae injected. In favor of the interpretation that the secretion of the larva provokes the reaction are: (1) the histologic evidence from the examination of noninfected lesions produced by the *T. akamushi;* [17] (2) the experimental observations of the cutaneous effects of the emulsified larvae; [18] and (3) our observations of the analogous reactions produced by the secretion of ticks. On the other hand, there is convincing inferential evidence that the rickettsiae play a rôle in the reactions. In the first place, as stated, lesions that resemble eschars and *taches noires* have been produced by the injection of rickettsiae of tsutsugamushi disease and of fièvre boutonneuse. Secondly, there is evidence of a phlebitic reaction at the base of the eschars, away from the site of suppuration, that is identical with the phlebitic reaction in other organs and hence strongly indicates a response to rickettsiae or their products. Thirdly, we have observed the organisms in the eschar in one case, and the probability is great that they were present in other cases but not demonstrable by our methods. In other words, it appears reasonable to conclude that the reaction in the eschar is the summation of the effects of the rickettsiae *and* of the secretion of the mite. It is hoped that facilities will soon permit experimental studies of the rickettsial content of the eschars at various stages of the disease. It would be of further interest to determine the rapidity with which the organisms reach the regional lymph nodes.

Specificity of Typhus Nodule of Macule

The specificity of the typhus nodule of the macule has been repeatedly emphasized (Wolbach, Todd, and Palfrey,[19] Ceelen [20]). Such nodules are found not only in epidemic typhus and Rocky Mountain spotted fever but also in scrub typhus. However, we should prefer to qualify the impression of specificity to this extent: perivascular ac-

cumulations of mononuclear cells of this variety are found in the corium in many dermatoses; *e.g.*, lichen planus, parapsoriasis, toxic erythema. Moreover, the endothelial cells of the surrounded vessels in these dermatoses may be swollen and even hyperchromatic (Fig. 14). Unless the nodule is associated with thrombosis of the vessel, karyorrhexis of its endothelial or adjacent infiltrating cells, and granular or fibrinoid degeneration of its wall (Fig. 23), we should be reluctant to regard the lesion as specific. Moreover, diseases such as disseminated lupus erythematosus, periarteritis nodosa, erythema elevatum diutinum, and bacterial sepsis may present some of the vascular changes just mentioned, and accordingly deserve differential histologic consideration.

Lung

In over half of the cases of scrub typhus we noted various degrees of interstitial pneumonitis. In 13 per cent of the cases the reaction was intense (Fig. 27) and seemed to us indistinguishable from the pneumonitis of "Q" fever[8] (Fig. 36), from the pulmonary reaction seen occasionally associated with severe rheumatic carditis,[21] from the pneumonia of toxoplasmosis[22] (Fig. 29), and from the outspoken cases of so-called atypical or viral pneumonias.[23] Although we have failed to demonstrate the organisms in sections, there are reasons for believing that this reaction merits the designation "rickettsial pneumonia." Other observers have demonstrated the rickettsiae of tsutsugamushi disease in the alveoli and alveolar lining of experimental animals.[14] It is of inferential interest that the occasional, large hyperchromatic cell of the alveolar lining in the pneumonic lungs of scrub typhus (Fig. 28) has a direct counterpart in the large alveolar cell of the interstitial pneumonitis of toxoplasmosis, in which cell numerous parasites may be found (Fig. 29).

Slight to moderate degrees of interstitial pneumonitis were found in a few of our cases of epidemic typhus and in one case of Rocky Mountain spotted fever. We were unable to find descriptions of this finding in the observations of others, although the "interstitial changes" mentioned by Dawydowskie[24] may possibly represent the same lesion. In none of our cases did the intensity of the reaction approach that of the more florid cases of scrub typhus or of Lillie's cases of "Q" fever.[8]

Heart

One of the outstanding features of the cardiac status in each of the rickettsioses is the disparity between the severity of the myocarditis and the degree of cardiac insufficiency. The corollary to this impression is the apparent state of preservation of the myocardial fibers notwith-

standing the intensity of the interstitial infiltrate. However, we are not entirely certain that the muscle of the heart is as well preserved as a first evaluation might indicate. We are concerned by a type of fragmentation of myocardial fibers, especially those in relation to foci of edema and inflammation (Fig. 16). This finding is noted in each of the rickettsial diseases. It would seem hazardous to dismiss such changes as artifacts. It is acknowledged that patients who survive the illness do not show clinical evidence of cardiac involvement. Nevertheless, until post-mortem data are available on the state of such hearts years after recovery, the impression of complete clinical recovery might perhaps be tempered with these considerations: (1) Patients with a fine, scattered fibrosis of the myocardium may not manifest clinical evidence of cardiac damage; (2) Not all patients with scrub typhus develop a myocarditis (Table II), and it is therefore conceivable that a portion of the survivors may belong in this category; and (3) Adequate post-mortem studies of the myocardium months or years after recovery are not yet available. The fact is that it is not known whether or not the myocardium is restituted to *status quo ante.*

Sickling

Apparent sickling of red blood cells was observed in the sections of one or more organs, usually the kidney, in 13 per cent of the cases of scrub typhus, in 35 per cent of the cases of epidemic typhus (Figs. 40 and 84), and in 1 of the 12 cases of Rocky Mountain spotted fever. The patients with epidemic typhus were all native Egyptians. We do not have any information on the incidence of sickling in Egyptians although studies of the West African native reveal an average incidence of 20 per cent [25] The evidence of the sickling in scrub typhus and spotted fever was observed in isolated organs of patients, all of whom were recorded as being of the white race. No data are available on the *in vivo* occurrence of sickling in patients with typhus fever. If this slight degree of sickling of red blood cells truly represents a latent sickling tendency, then the incidence seems remarkably high.[26] Such focal sickling of red blood cells is seen occasionally in routine autopsy material of patients dying of a variety of causes. The impression is generally held that the phenomenon represents a post-mortem packing and distortion of cells. However, it must be noted that individual, isolated cells in these foci assume a sickled shape (Fig. 40). The pathogenesis of such distortion of erythrocytes cannot be considered settled. Therefore, because the significance of the observation is not clear, and because of the possibility, at least, that it may reflect local anoxia, the finding is deemed worthy of record and comment.

Rickettsiae in Tissues

There was available to us only a single smear from cases of scrub typhus. This one smear was of spleen and with the Giemsa stain (Wolbach modification [27]) numerous rickettsiae were found (Fig. 44). A systematic search for rickettsiae in sections was made in only 6 cases of scrub typhus, the tissues of which were fixed in Regaud's solution. Intracytoplasmic endothelial bodies, which we are confident are rickettsiae, were found in only one section—that of an eschar. We were, however, unable to obtain a satisfactory photograph for purposes of demonstration in this paper. It is clear that rickettsiae are discovered with far greater ease in the sections of Rocky Mountain spotted fever and louse-borne typhus, than in tsutsugamushi disease. Of importance in facilitating the detection of organisms is the cutting of sections of not more than 4 μ in thickness, as well as proper fixation and staining.[27] At best, the unmasking of rickettsiae in the tissues of scrub typhus is currently an unreliable procedure.

Kidney

Apart from focal interstitial nephritis, cloudy swelling and vacuolization of the tubular epithelium, and occasional vascular alterations, the kidneys of the rickettsioses have been described in the literature as showing few significant changes. The glomeruli are usually dismissed as being essentially normal. For example, Kawamura,[17] in his exceedingly able and comprehensive discussion of tsutsugamushi disease, did not mention glomerular damage. Kouwenaar,[28] in his study of Sumatran mite typhus, stated that "for the most part the glomeruli are intact; occasionally there is definite capsular adhesion." In their classic study of epidemic typhus, Wolbach, Todd, and Palfrey [19] found glomerulonephritis in 1 of 37 cases, and intracapillary proliferation in 9 others. Ceelen [20] found no glomerular alteration in his cases of epidemic typhus. Munk [29] mentioned the finding of "Infektnephritis" in instances of epidemic typhus in which hematuria was present without significant glomerular alteration. In Rocky Mountain spotted fever, Wolbach [27] found essentially normal glomeruli except for an increased cellularity in 1 of the 5 cases. In Lillie's series of cases of spotted fever,[13] the glomeruli were not remarkable. Several conspicuous exceptions are found in the literature and these are in connection with *epidemic typhus.* Caffarena [30] found acute diffuse glomerulonephritis in 67.5 per cent of his cases. Schopper [31] observed swelling and hyperplasia of glomerular endothelial cells and suggested that his cases might represent the earliest hemorrhagic phase of glomerulonephritis. Dawydowskie [24] noted a focal destructive glomerulitis in one-third of his

series of cases of louse-borne typhus. Wetzel,[32] in describing a case of epidemic typhus with glomerulonephritis, insisted that too little emphasis was being paid to this lesion.

The interpretation of the glomerular changes in the current material approximates the conclusions of Caffarena.[30] We found acute diffuse glomerulonephritis * in 30 per cent of the cases of scrub typhus, 78 per cent of epidemic typhus, and 50 per cent of Rocky Mountain spotted fever (Figs. 52 to 56, and 83). Our data did not permit of a systematic correlation of the histologic changes with alterations in the chemical constituents of the blood. It is hoped that detailed correlative studies will soon be forthcoming. The glomerular lesions in the three rickettsial diseases are basically similar. They represent the intracapillary form of acute diffuse glomerulonephritis, characterized by swelling and hyperplasia of endothelial cells, normal or slightly thickened, sometimes duplicated, occasionally hyalinized, capillary basement membranes, and marked ischemia of the tufts. Intracapillary hyaline fibers[32a] were not present, but in our experience absence of such fibers does not preclude the existence of glomerulonephritis with impairment of renal function. The high incidence of acute diffuse glomerulonephritis (78 per cent) in the current series of cases of epidemic typhus is in harmony with the recent observation made by Yeomans, Snyder, Murray, Zarafonetis, and Ecke[33] who noted clinically that typhus fever frequently produced severe impairment of renal function.

Pathogenesis of Glomerulonephritis. If the existence of glomerulonephritis is granted, two questions arise: (1) Is the glomerulonephritis, *i.e.*, the injury to the glomerular capillaries, due to the direct action of the rickettsiae? (2) What are the chances of restoration of the glomeruli to normal? We have not found organisms in the glomeruli in any of the typhus fevers, nor do we know of any such observation. It seems unreasonable to assume that organisms would lodge directly in the endothelium of glomerular capillaries and provoke a fairly uniform response throughout the kidney. The damage to vessels in other organs in which rickettsiae are found is characteristically varied in intensity. Therefore the glomerular alteration is regarded as a remote effect of the rickettsiae—either hyperergic or toxic—in much the same sense that acute diffuse glomerulonephritis following scarlet fever is attributed to the distant or secondary effects of *Streptococcus haemolyticus*. As we understand them, this is essentially the view of Julliard and Henaff.[34]

Restitution of Glomeruli. Notwithstanding the development of glomerulonephritis, it appears that the chances for anatomic restora-

* Although we are confident that these cases represent an early acute diffuse glomerulonephritis, we are aware that some pathologists may not concur with this interpretation in all instances.

tion of the glomeruli are encouraging. Disturbance in the basic architecture of the glomeruli is infrequent. The basement membranes are usually not appreciably affected, notwithstanding the serious endothelial changes and the resulting ischemia of the capillaries. The cloudy swelling and often marked hydropic vacuolization of the proximal convoluted tubules are entirely reversible. The focal interstitial inflammation that is usually present will either resolve or cause cicatrization, depending on its severity, but it is not likely to constitute a source of significant impairment of renal function in the future. Finally, an analogy is afforded by the resolution of acute diffuse glomerulonephritis that undoubtedly occurs in many cases following recovery from bacterial infections.[35]

Circulatory Failure

There has been considerable discussion in the literature regarding the pathogenesis of the circulatory collapse of patients with typhus fevers. The rôles of the myocardium and of the central nervous system have been emphasized. However, in the rickettsioses, no less than in many instances of bacterial sepsis, the decisive, terminating clinical effects are a product of the disturbances of more than one organ. In view of the histologic changes, it would seem justified to consider the contributory effects particularly of the heart, the central nervous system, the lungs, the kidneys, and the adrenals. The heart has been discussed. The findings in the central nervous system are impressive, but we have not been able to study the vital centers systematically. Therefore we do not have the detailed data that would be necessary for an evaluation of this phase of the problem. From studies of the nervous system in epidemic typhus,[36] and from our own inadequate observations, there is reason to believe that the nervous system reacts to a degree comparable to that seen in other organs.

Circulatory collapse occurs commonly without significant involvement of the lungs, but it is interesting that there is more or less parallelism between the intensity of the interstitial pneumonitis and of the myocarditis. Surely, pneumonitis, when severe, contributes to the circulatory load. The kidneys, as stated, are frequently involved and undoubtedly contribute to the hemodynamic burden, and to the death of the patient, but it is to be emphasized that circulatory collapse may occur without significant, histologically evident, renal damage. The expression "histologically evident" is used advisedly because of the possibility that the adrenal gland, for example, may mediate its vital control of the electrolytes of the blood through the kidneys without provoking microscopically visible clues.[37] The adrenal gland is mentioned at this point and in this connection because we have observed

the same type of "tubular degeneration" of the cortex in rickettsial disease as Rich [10] has recently noted in cases of bacterial sepsis (Fig. 49). Rich suggested that this degeneration may be related to the production of shock in septicemias. It is pertinent to recall that less than a decade ago, considerable routine importance was attached to the rôle of the adrenal cortex in the genesis of shock in infectious states. At that time, adrenal cortical therapy was regarded as effective by some [38, 39] and ineffective by others.[40] Possibly the lack of convincing histologic support discouraged prolonged interest, which may now be renewed with the impetus of Rich's observation. Undoubtedly, this change in the cortical cells is regarded by many as post-mortem degeneration, an opinion strengthened, perhaps, by the susceptibility of the inner zone to post-mortem liquefaction. However, the "tubular degeneration" to which we refer occurs in the outer zones and we are convinced that the lesion is not an artifact, whatever its physiologic implications may be. The fact is that shock with vasomotor atony and collapse, hypochloremia, azotemia, and changes in blood volume are present in the severe cases of rickettsial infection. The further fact is that these are precisely the changes associated with adrenal insufficiency. The data at hand do not permit drawing a complete comparison, but the parallel is, at the least, suggestive. We should be reluctant to adopt the mechanistic explanation for the vascular atony and increased permeability that occur in epidemic typhus and which have been attributed to actual damage to the vessels produced directly by the rickettsiae.[41] To be sure, such intrinsic vascular damage is often evident in louse-borne typhus, but it is usually lacking in scrub typhus, that is, unless it is inferred from the perivascular infiltrate that the vessel concerned has been altered, an inference by no means necessarily justified. Moreover, notwithstanding the difference in morphologic evidence of vascular injury, the severity of vasomotor collapse appears just as striking in scrub typhus as in epidemic typhus. It would, therefore, seem more likely that this profound hematic and vasomotor disturbance is mediated by a universally acting, humoral mechanism rather than by local organic changes of various stages and degrees. The rôle of the adrenal cortex in this physiologic upheaval and the therapeutic trial of cortical extract is again suggested for consideration.

The Cellular Infiltrate

The quality of the cellular infiltrate in all of the organs of the rickettsioses is basically similar and consists essentially of lymphocytes, plasma cells, and large basophilic as well as acidophilic macrophages. The large basophilic macrophage has repeatedly been singled

out for comment in discussions of the rickettsial diseases. It has been variously labelled Türk cell, large lymphocyte, endothelial phagocyte, etc. It is discussed here because it may throw light on one aspect of the pathogenesis of the lesions in the typhus fevers.

These cells are produced in abundance in the spleen following the repeated injection of foreign serum; [42] indeed, the cytologic picture of such spleens simulates that in typhus fever. The evidence in both experimental and autopsy material suggests that the cells belong to the lymphatic series. Transitions from the small lymphocyte, with basophilic cytoplasm and pachychromatic nucleus, to the large basophilic macrophage and thence to the leptochromatic, acidophilic macrophage have been observed by numerous investigators (Huebschmann,[43] Tschaschin,[44] Maximow,[45] Bloom,[46] Taliaferro and Mulligan,[47] and Kolouch [48]). In other words, the *hematogenous* macrophage may become indistinguishable from the *histogenous* macrophage. Moreover, this large basophilic macrophage, or, "acute splenic tumor cell," has been shown most graphically by Rich, Lewis, and Wintrobe [42] to have the characteristics of a lymphoblast, an opinion, as they stated, which was previously expressed by Huebschmann.[43] It is of considerable pathogenetic interest that this cell is found in abundance in allergic reactions.[42] Moreover, this evolution of the lymphocyte helps to explain the concentration of various stages of the series of mononuclear cells in veins of many organs (Fig. 57). Apparently, these large basophilic macrophages have been observed by Rabinowitsch to the extent of 0.5 to 10 per cent of the white blood cells in cases of epidemic typhus.[49] It is suggested that detailed studies of these cells in the typhus fevers be made from the peripheral blood as well as from smears of organs at autopsy. Information concerning the time of appearance and disappearance of these cells from the blood might be revealing.

Hyperergy

While we are aware of the burden that is prone to be attached to an interpretation of the genesis of a histologic change in terms of allergy, nevertheless, certain alterative changes in the rickettsioses are so striking as to merit discussion. These include: (1) necrosis of lymph nodes and spleen, simulating responses to known allergens, (2) the predominance of plasma cells and especially of large basophilic macrophages, which are known to participate in allergic reactions [42] (eosinophilic leukocytes were inconspicuous), (3) the glomerulonephritis, (4) the mononuclear cell infiltration and edema of the mural and valvular endocardium as well as of the intima of arteries (Fig. 19). This reaction is seen not only in sepsis generally, but following sensitization to

serum.[7] (5) Finally, there is the pronounced fibrinoid degeneration of the interstitial collagen of the myocardium (Fig. 17), of the vessels (Fig. 22), and of the perivascular tissue (Fig. 25). This type of alteration has long been identified with hyperergic responses.[50-52] The same type of fibrinoid alteration is observed in periarteritis nodosa, in disseminated lupus erythematosus, and in the vascular damage following sensitization to the sulfonamides.[53,54] In short, these various reactions, considered together, appear to us to present strongly presumptive histologic evidence of "altered tissue reactivity" or one phase of allergy. In this regard, it may be recalled that the phenomena of allergy apply to the rickettsiae as well as to bacteria (Mudd [55]).

Pathologic Concept of the Rickettsioses

From the pathologic point of view, the rickettsioses have long been regarded as a form of diffuse vascular disease. Surely, this impression is almost inescapable after a study of epidemic typhus and spotted fever. However, the histology of scrub typhus may perhaps warrant a change in the direction of emphasis. Although focal, more or less bland thrombophlebitis in scrub typhus is not uncommon, actual arteritis occurs rarely, and, in our series, was never of the fibrinoid variety seen in louse-borne or tick-borne typhus. Moreover, the arteritis of scrub typhus does not seem to be a lesion *sui generis,* but, rather, appears to be secondary to an extension of the periarterial infiltrate into the wall. This interpretation was made previously by Kouwenaar.[28] Yet, notwithstanding the disparity in the histologic evidences of vascular damage, there are basic clinical, etiologic, and, in many respects, immunologic similarities between scrub typhus and the other rickettsioses. Therefore, perhaps a re-evaluation of the significance of the pathologic changes is in order. A close analogy to this problem is found in a nonrickettsial disease—acute disseminated lupus erythematosus (Libman-Sacks disease). The prominence of the degeneration of vessels in many organs led initially to the concept that this entity was a diffuse vascular disease. However, further studies prompted a broader concept; namely, that disseminated lupus erythematosus was in effect a disturbance of collagen, be it of a vessel, a cardiac valve, or a serous membrane. Moreover, the histologic and clinical pictures were such as to suggest a hyperergic reaction.[52, 56, 57] The analogy may be extended by reference to periarteritis nodosa and to the arteritis that follows administration of sulfonamides. In other words, in the over-all view of the pathologist, the more remote, possibly hyperergic effects of the rickettsiae—the effects on the adrenal gland, on the glomeruli, and on the production of interstitial inflammation—assume

more importance than the direct damage wrought by the localization of the rickettsiae.

Summary and Conclusions

1. The histologic preparations and protocols of 78 cases of scrub typhus (tsutsugamushi disease), 24 cases of epidemic (louse-borne) typhus, 12 cases of Rocky Mountain spotted fever, and the sections of lungs of 2 cases of American "Q" fever were studied.

2. The primary lesion, or eschar, is considered to be provoked by the combined action of the secretion of the larval mite and the inoculated rickettsiae. It is suggested that the absence of the eschar in certain instances of scrub typhus may be due to variations in cutaneous immunity.

3. Interstitial pneumonitis of a marked degree is common in scrub typhus in contrast with epidemic typhus and Rocky Mountain spotted fever. The histologic picture of the interstitial pneumonitis of scrub typhus is indistinguishable from that of "Q" fever, rheumatic fever, toxoplasmosis, and viral pneumonia.

4. It is concluded that the amount of hepatic damage as noted histologically does not warrant the presumption that hypoproteinemia is due to hepatic insufficiency.

5. Early, acute, diffuse glomerulonephritis is common in scrub typhus, epidemic typhus, and Rocky Mountain spotted fever. The indirect rôle of the rickettsiae in the pathogenesis of the glomerulonephritis is indicated.

6. The focal encephalitis or nodule of scrub typhus is qualitatively similar to that of epidemic typhus and is in contrast to the "microinfarct" of Rocky Mountain spotted fever. The nodules of scrub typhus and epidemic typhus are practically limited to the gray matter, whereas the encephalitis of spotted fever involves the white matter preponderantly.

7. Contrary to the generally held impression, there is a sparsity of histologically evident vascular damage in scrub typhus. Arteritis is exceedingly slight in scrub typhus in contrast with epidemic typhus and Rocky Mountain spotted fever. Accordingly, it is suggested that the designation "diffuse vasculitis" when applied to scrub typhus represents an oversimplification not justified by the morphologic evidence.

8. It is concluded that the peripheral circulatory failure in patients with rickettsial diseases is a complex phenomenon which cannot be explained solely on the basis of morphologic damage of vessels. The contributory rôle of the adrenal gland in the circulatory failure is suggested.

9. The evidence of lymphoblastic origin for the cells characterizing the interstitial infiltrate is presented. The identification of the large "basophilic macrophage" with the "acute splenic tumor cell" is suggested and the evidence pointing toward the association of these cells with an allergic response is given.

10. Reasons are presented for regarding the rickettsial diseases from a broader pathologic point of view than that of diffuse vascular diseases. Emphasis is placed on the importance of the indirect, possibly toxic, but more likely hyperergic effects of the rickettsiae, on the basis of certain histologic responses which are regarded as strongly presumptive evidence of the action of allergens. These responses include fibrinoid degeneration of collagen, the necrosis of lymph nodes and spleen, the predominance of the basophilic macrophage and associated cells, and the acute diffuse glomerulonephritis.

The photomicrographs were taken by Mr. Roy M. Reeve of the Army Institute of Pathology. His skillful cooperation is gratefully acknowledged.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 99

FIG. 1. Acc. 101690. Eschar of scrub typhus removed at autopsy. Duration of illness, 17 days. The superficial mass of necrotic epidermis, karyorrhectic cells, blood, serum, and bacteria represents the black crust that was about to be sloughed. Hematoxylin and eosin stain. × 20. Neg. 83436.

FIG. 2. Acc. 112440. Eschar of scrub typhus removed at autopsy. Duration of illness, 15 days. The residual epidermis at the base of the lesion indicates the initial intra-epidermal location of the pustule. Hematoxylin and eosin stain. × 50. Neg. 83399.

FIG. 3. Acc. 112440. Higher magnification of one of a series of sections from the lesion in Figure 2. The amorphous nature of the pustular contents, the distortion of the inflammatory cells, and the irregular acanthosis may be seen. Hematoxylin and eosin stain. × 145. Neg. 82918.

FIG. 4. Acc. 101690. Section from angle of eschar showing the intra-epidermal position of the pustule, its parakeratotic roof, the peripheral acanthosis, and vacuolization of the cells of the rete malpighii. Hematoxylin and eosin stain. × 145. Neg. 83153.

FIG. 5. Acc. 101690. Section of epidermis at the periphery of eschar showing parakeratosis, acanthosis, edema, hyperchromatism, and disturbance in polarity of cells of basal layer and lower stratum spinosum. Hematoxylin and eosin stain. × 500. Neg. 77500.

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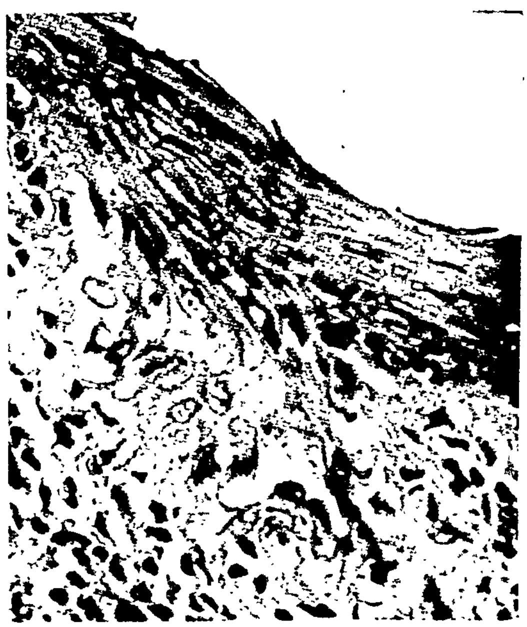


5

Allen and Spitz Comparative Study of Scrub Typhus

PLATE 100

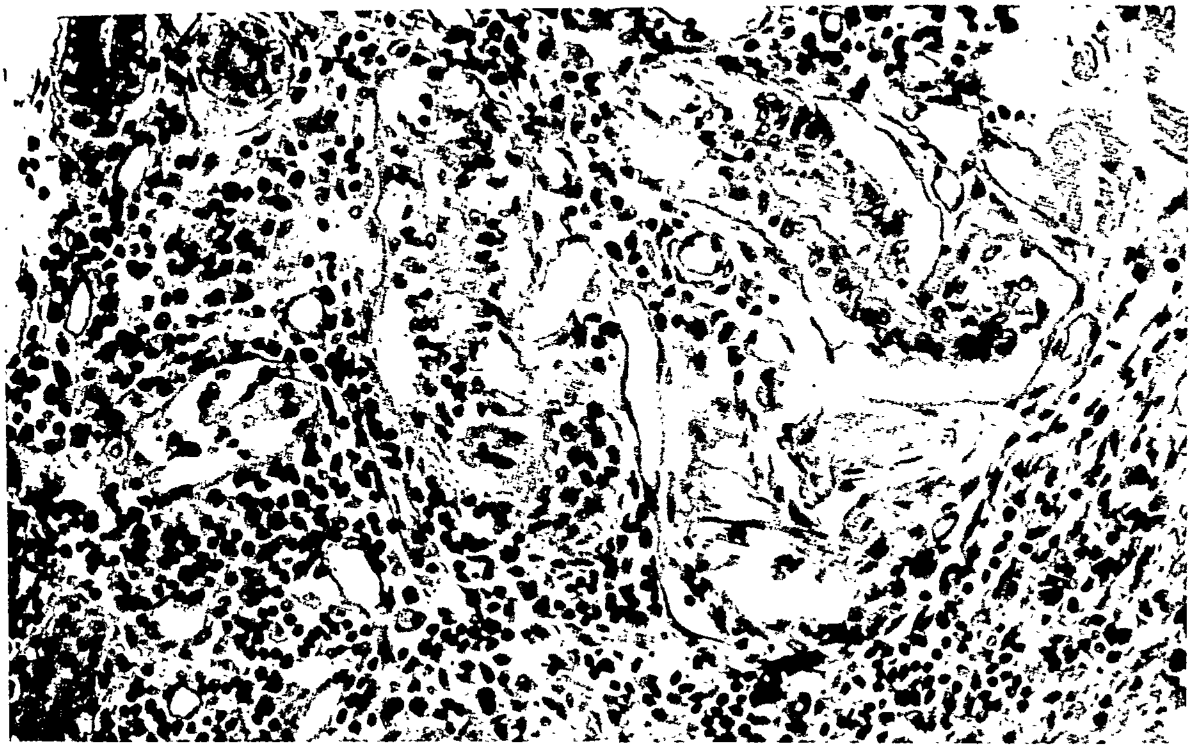
FIG. 6. Acc. 112440. Section of mid-corium shows the mononuclear character of the infiltrate and the localization about the coils of sweat glands. Hematoxylin and eosin stain. ×280. Neg. 83144.

FIG. 7. Acc. 102989. The infiltrate in this section of skin produced by a sterile tick bite matches in quality that of the eschar except for the abundant eosinophilic leukocytes in the former. Hematoxylin and eosin stain. × 280. Neg. 83150.

FIG. 8. Acc. 112440. Foreign body reaction in the corium of an eschar. A small fragment of mite, not apparent in this section, was found at the periphery of this lesion. Hematoxylin and eosin stain. × 120. Neg. 83143.

FIG. 9. Acc. 101690. Section taken from the lower reticular layer of the corium. away from areas of suppuration, showing mononuclear infiltration of the intima. This reaction, and that shown in Figure 10, represent the type of phlebitis found in other organs and is presumed to be due to the effect of rickettsia rather than to a nonspecific contiguous inflammation. Hematoxylin and eosin stain. × 145. Neg. 82921.

FIG. 10. Acc. 101690. Section showing phlebitis at junction of dermis and hypodermis of eschar. The granular type of intimal degeneration and the various stages in the formation of intimal verrucae may be noted. Hematoxylin and eosin stain. × 145. Neg. 83152.



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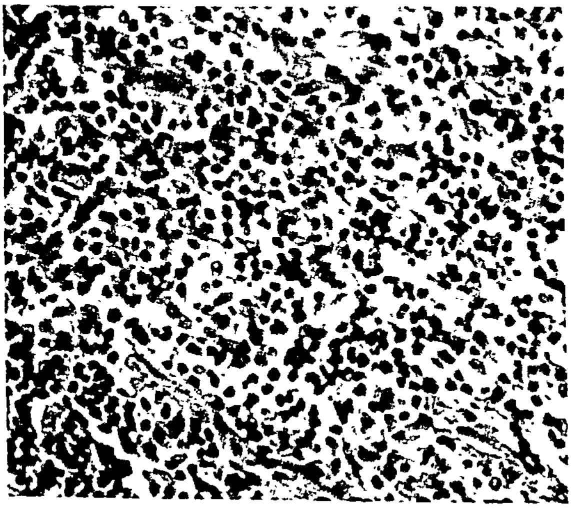

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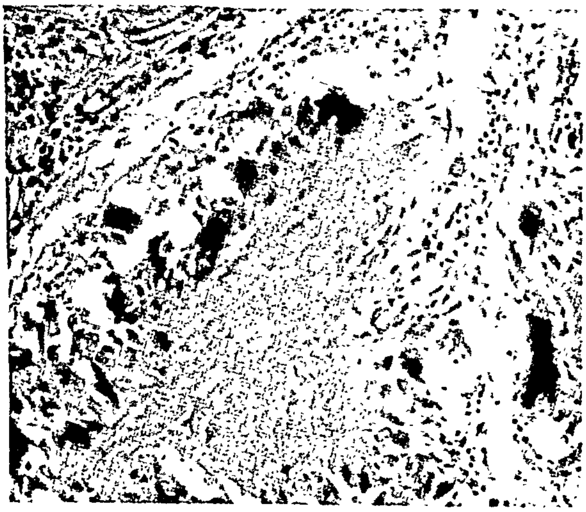

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Plate 101

Fig. 11. Acc. 112281. Macule of scrub typhus. Duration of rash, 7 days. There is a perivascular infiltration of mononuclear cells forming the equivalent of the "typhus nodule." Hematoxylin and eosin stain. × 145. Neg. 82911.

Fig. 12. Acc. 112525. Macule of scrub typhus. Duration of rash, 7 days. Of note is the edema of the papillary layer, the platelet thrombus, and the swelling and hyperchromasia of the endothelial cells of a venule. Hematoxylin and eosin stain. × 230. Neg. 82912.

Fig. 13. Acc. 105720-8A. Macule of epidemic typhus. Duration of rash, 13 days. The eccentrically perivascular "typhus nodule" and the thrombophlebitis are illustrated. Hematoxylin and eosin stain. × 230. Neg. 82913.

Fig. 14. Acc. 119287. Section of dermis from a case of lichen planus showing perivascular infiltration of lymphocytes, plasma cells, and macrophages as well as endothelial swelling. This nonspecific lesion simulates the "typhus nodule" but lacks endothelial karyorrhexis and thrombosis. Hematoxylin and eosin stain. × 500. Neg. 82970.

Fig. 15. Acc. 112435. Macule of scrub typhus. Duration of rash, 8 days. This photograph illustrates a type of degeneration of sweat glands seen not only in the various typhus fevers but in many dermatoses. Hematoxylin and eosin stain. × 120. Neg. 83159.

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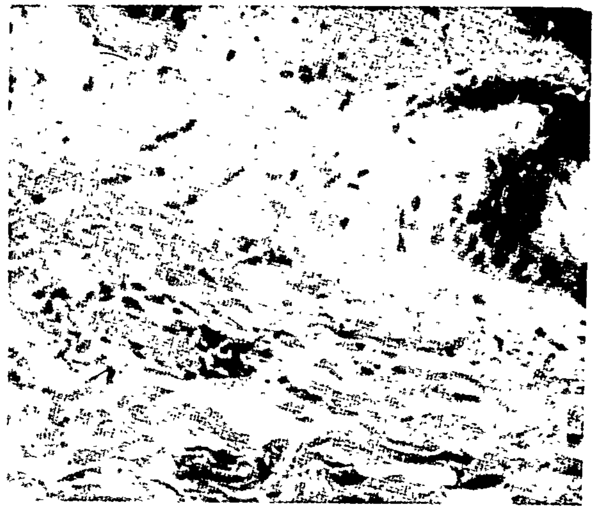

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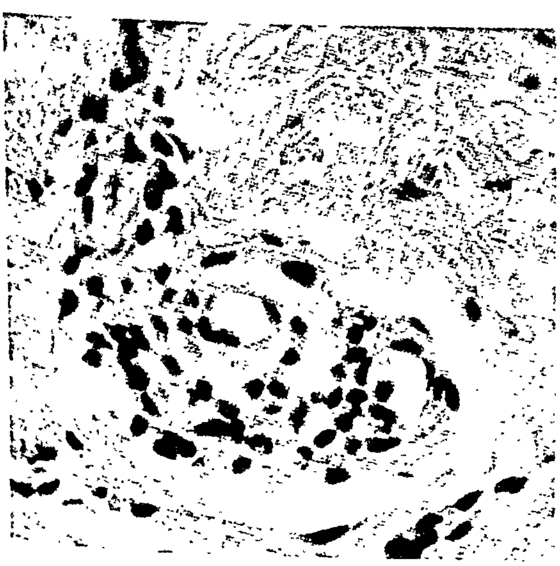

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PLATE 102

FIG. 16. Acc. 112440. Interstitial myocarditis of scrub typhus, showing (1) the interfibrous distribution, (2) the various types or stages of development of mononuclear cells, (3) the interstitial edema, and (4) the apparent degenerative changes in the muscle fibers. Hematoxylin and eosin stain. $\times$ 500. Neg. 83149.

FIG. 17. Acc. 112451. Interstitial myocarditis of scrub typhus with fibrinoid degeneration of collagen that strikingly simulates the interstitial lesion of the myocardium of disseminated lupus erythematosus (Fig. 18). Hematoxylin and eosin stain. $\times$ 750. Neg. 83147.

FIG. 18. Acc. 87549. Interstitial myocarditis of disseminated lupus erythematosus showing the type of fibrinoid degeneration almost pathognomonic of this disease. Hematoxylin and eosin stain. $\times$ 375. Neg. 83148.

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PLATE 103

FIG. 19. Acc. 112281. Heart of scrub typhus showing marked infiltration of mural endocardium by various mononuclear cells. Similar infiltrations occur beneath endothelium of valves, coronary arteries, and aorta. Hematoxylin and eosin stain. × 250. Neg. 83016.

FIG. 20. Acc. 112451. Ischemic type of degeneration of myocardium of scrub typhus. Hematoxylin and eosin stain. × 230. Neg. 82900.

FIG. 21. Acc. 112525. Intramyocardial neuritis in scrub typhus. Hematoxylin and eosin stain. × 280. Neg. 82924.

FIG. 22. Acc. 105720-22A. A lesion of the type found in periarteritis nodosa in the myocardium of a patient with epidemic typhus. No eosinophilic leukocytes are present. Hematoxylin and eosin stain. × 230. Neg. 80318.

FIG. 23. Acc. 105720-21A. "Typhus nodule" of myocardium in epidemic typhus. showing hyperchromasia and swelling of endothelium of arteriole, granular degeneration of its wall, and karyorrhexis of cells. Hematoxylin and eosin stain. × 600. Neg. 78554.

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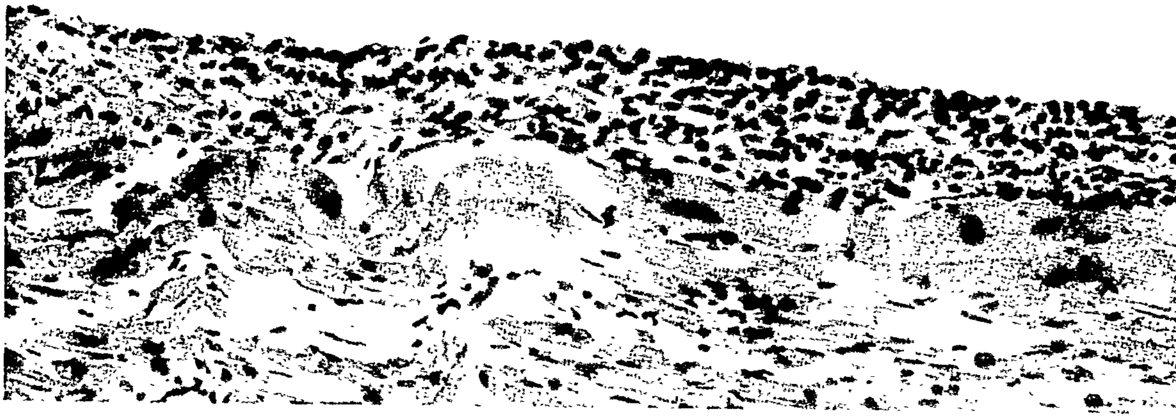

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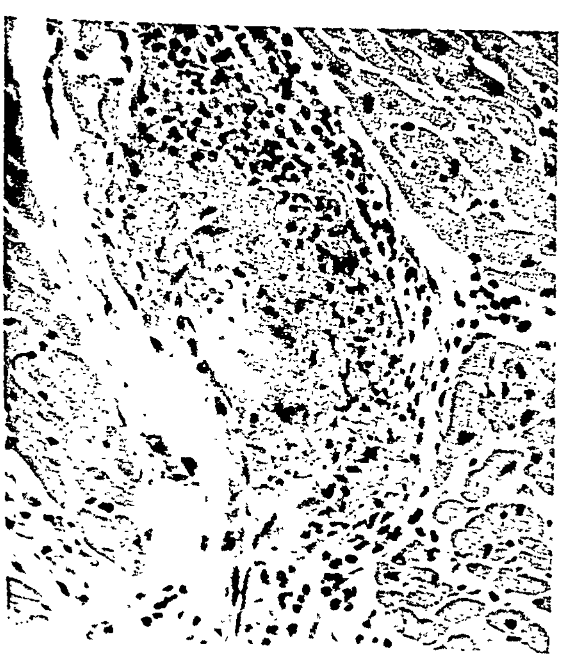

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Comparative Study of Scrub Typhus

PLATE 104

FIG. 24. Acc. 94537. Interstitial infiltrate of myocardium in scrub typhus showing lymphocytes, plasma cells, basophilic macrophages, and several Anitschkow myocytes. Hematoxylin and eosin stain. × 705. Neg. 82932.

FIG. 25. Acc. 105720-22A. Epicardium of louse-borne typhus showing periarterial fibrinoid change, again of the type seen in disseminated lupus erythematosus. Hematoxylin and eosin stain. × 250. Neg. 83195.

FIG. 26. Acc. 113898. Hemorrhagic tracheitis in scrub typhus. Hematoxylin and eosin stain. × 145. Neg. 82920.

FIG. 27. Acc. 113446. Interstitial pneumonitis of scrub typhus showing edema and mononuclear cell infiltration of septa and alveoli. Hematoxylin and eosin stain. × 120. Neg. 82723.

FIG. 28. Acc. 111595. Interstitial pneumonitis of scrub typhus showing not only the septal involvement but particularly the enlarged, hyperchromatic alveolar "epithelial" cells. Hematoxylin and eosin stain. × 500. Neg. 82730.

FIG. 29. Acc. 97029. Interstitial pneumonitis of toxoplasmosis showing a picture similar to Figure 28. The protozoan parasites are present in the largest alveolar lining cell included in this photomicrograph. Hematoxylin and eosin stain. × 330. (Material obtained through the courtesy of Dr. Henry Pinkerton, St. Louis University, School of Medicine.) Neg. 82904.

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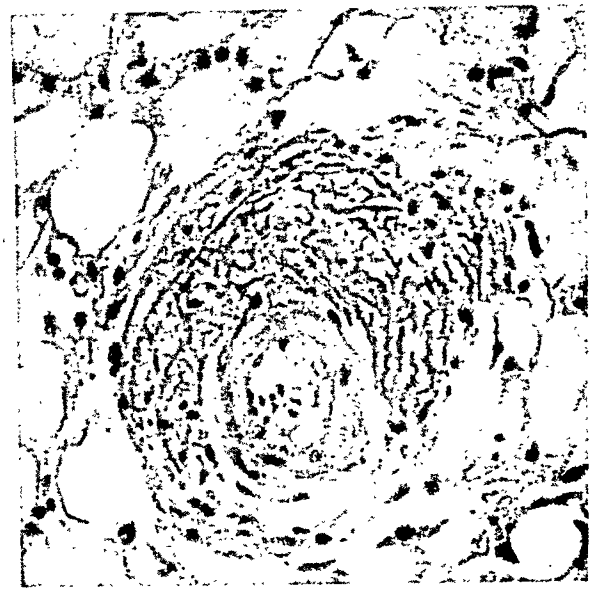

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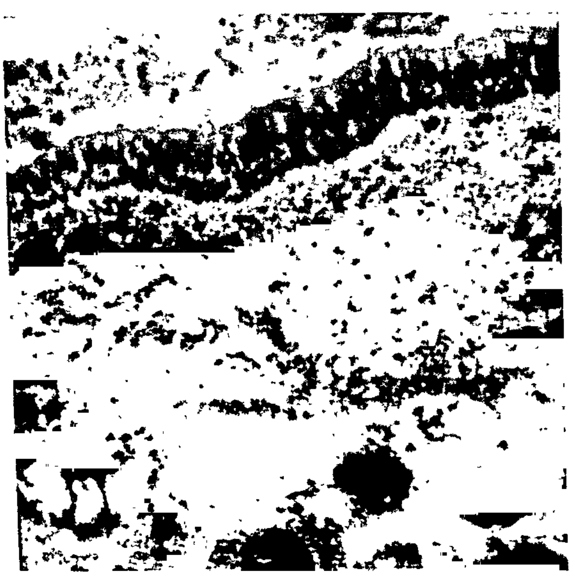

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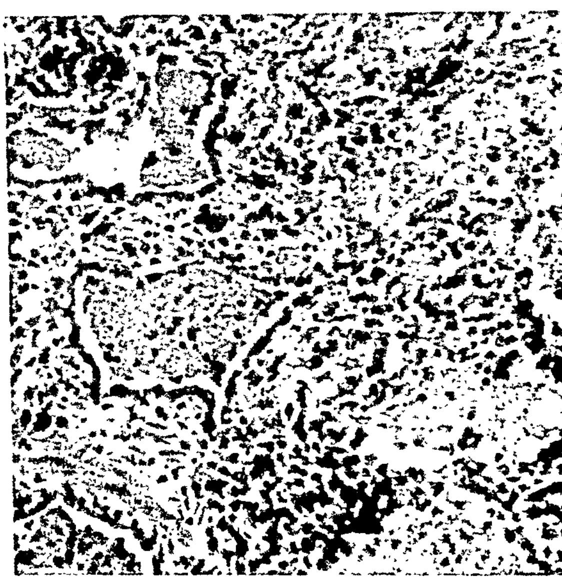

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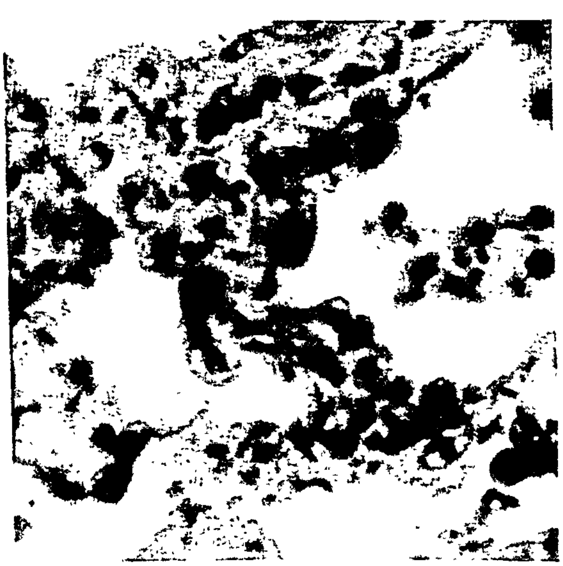

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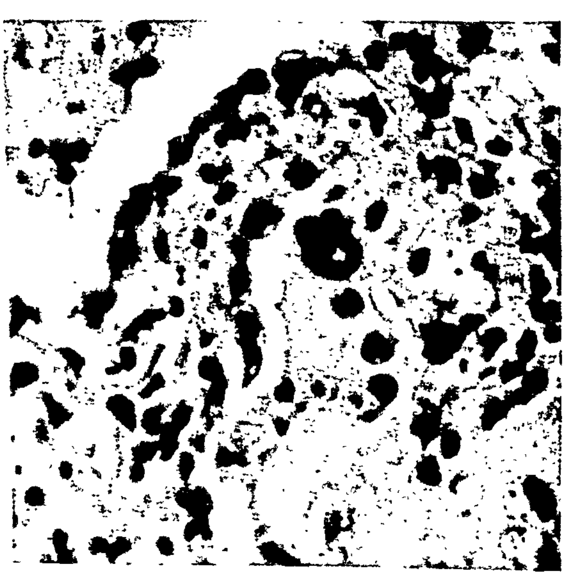

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Plate 105

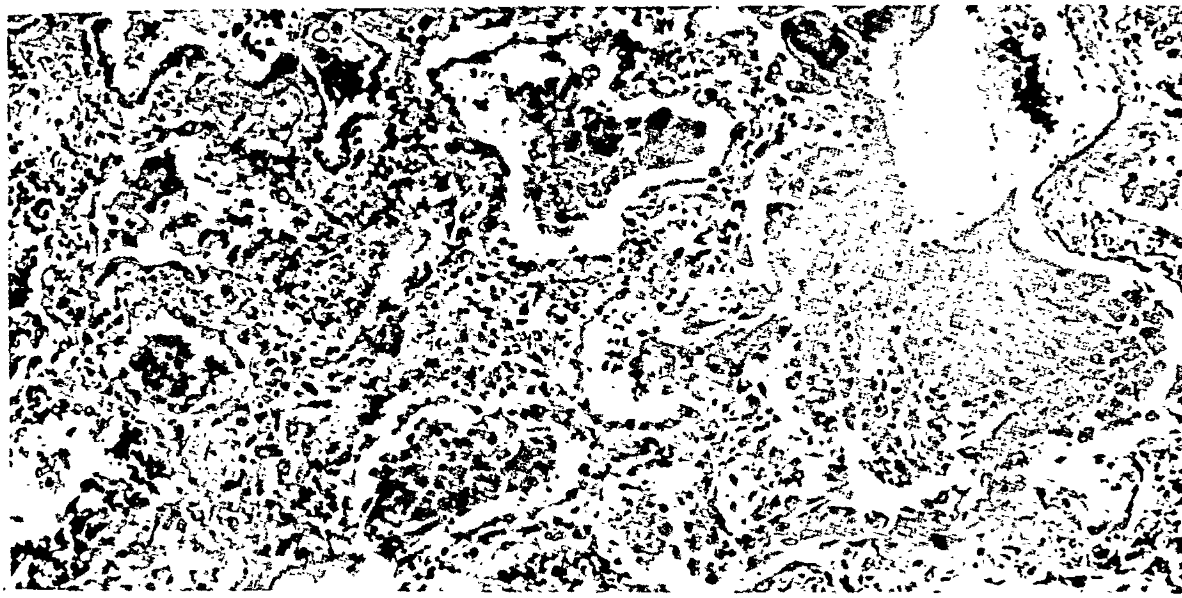
Fig. 30. Acc. 112304. Interstitial pneumonitis of scrub typhus showing (1) septal edema and mononuclear cell infiltration, (2) macrophages and partially organized alveolar exudate, (3) prominent alveolar lining. Hematoxylin and eosin stain. × 145. Neg. 82718.

Fig. 31. Acc. 112240. Interstitial pneumonitis of scrub typhus illustrating the septal edema and relative ischemia, as well as the hemorrhagic exudate. Hematoxylin and eosin stain. × 230. Neg. 82724.

Fig. 32. Acc. 112240. Interstitial pneumonitis of scrub typhus showing edema and mononuclear cell infiltration of an interlobar septum. The alveolar lining is prominent. Hematoxylin and eosin stain. × 120. Neg. 82729.

Fig. 33. Acc. 94655. Phlebitis in lung in case of scrub typhus, showing characteristic intimal mound of mononuclear cells. This type of phlebitis is seen commonly in the kidneys of scrub typhus. Hematoxylin and eosin stain. × 120. Neg. 82728.

Fig. 34. Acc. 112261. Pleuritis accompanying interstitial pneumonitis of scrub typhus. The mononuclear cells suggest a mesothelial origin. Hematoxylin and eosin stain. × 145. Neg. 82719.



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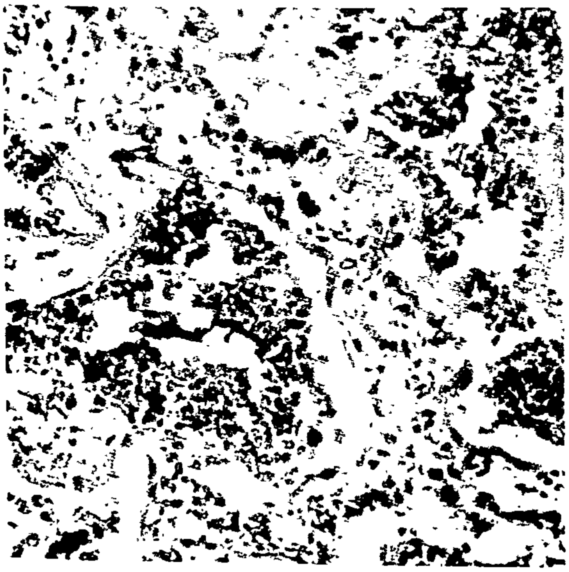

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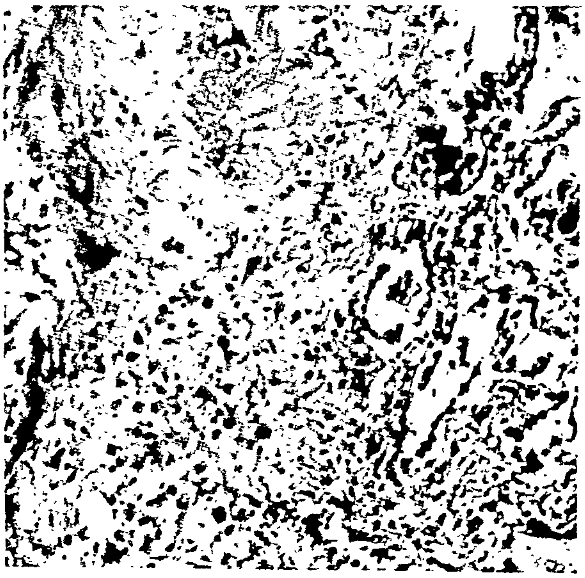

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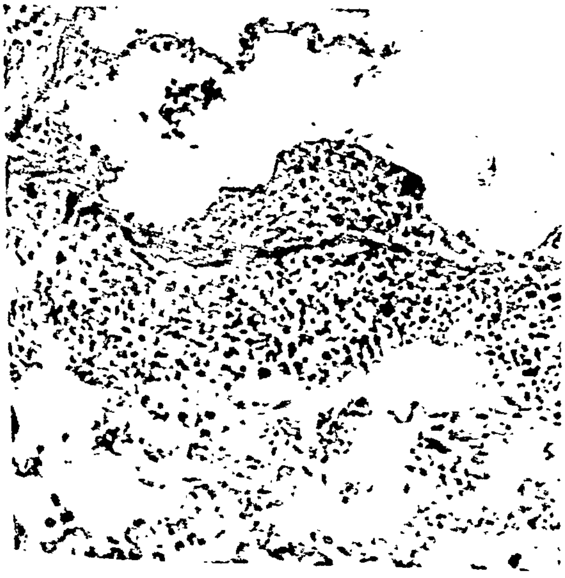

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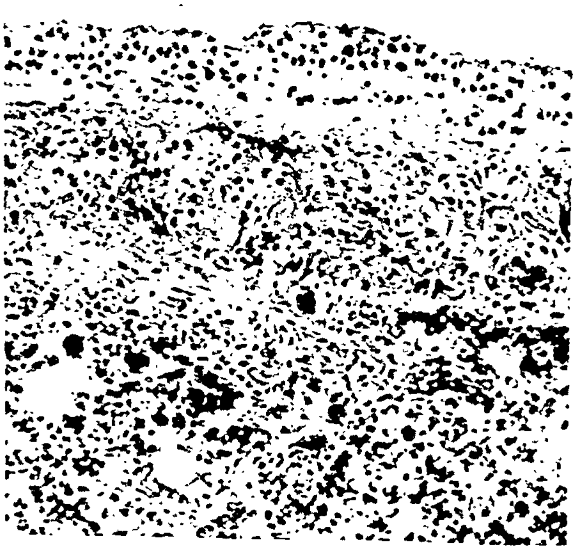

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PLATE 106

FIG. 35. Acc. 105720-14A. Interstitial pneumonitis in a case of epidemic typhus. Hematoxylin and eosin stain. × 115. Neg. 83188.

FIG. 36. Acc. 104973. Interstitial pneumonitis of American "Q" fever, with marked bronchiolitis and mononuclear cell infiltration of the septa and alveoli. This pneumonitis is similar to that observed in scrub typhus. Hematoxylin and eosin stain. × 145. Neg. 82975.

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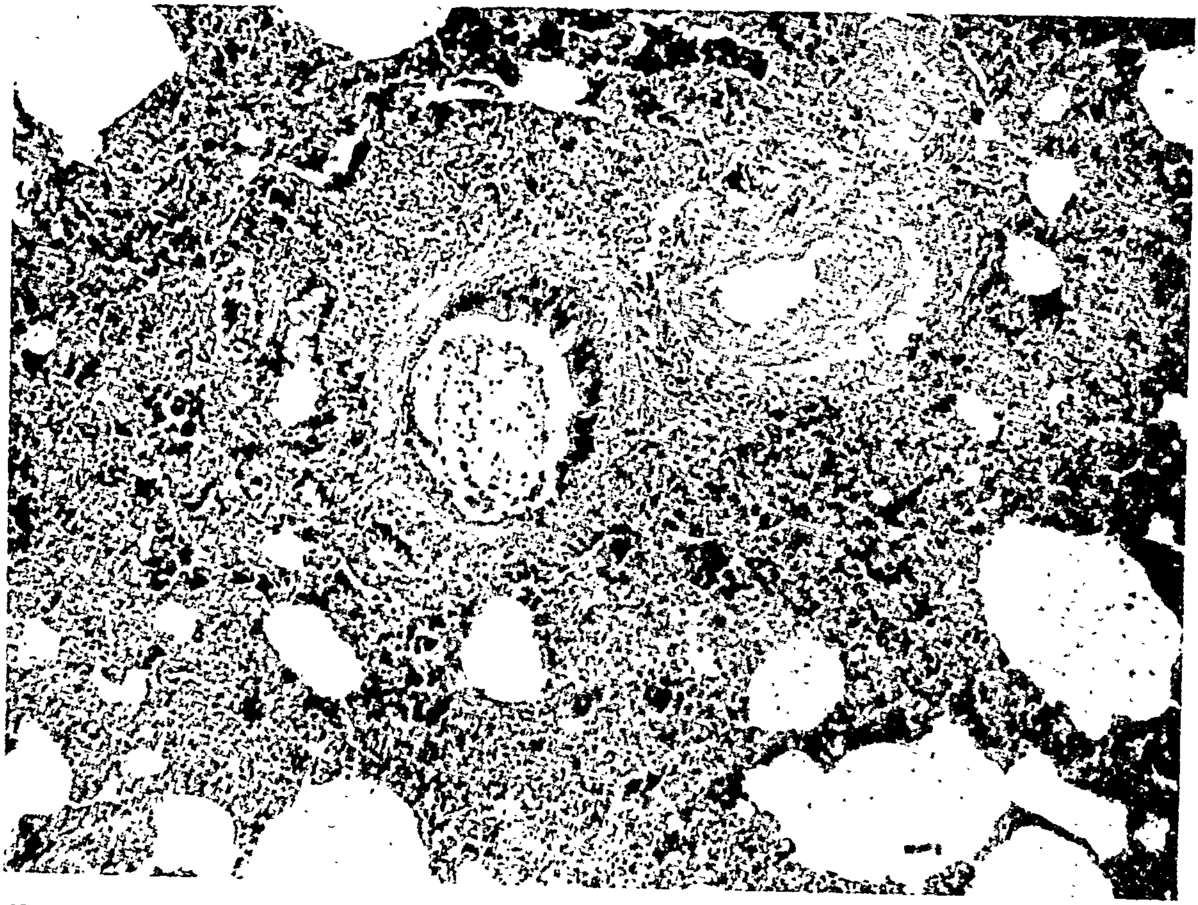

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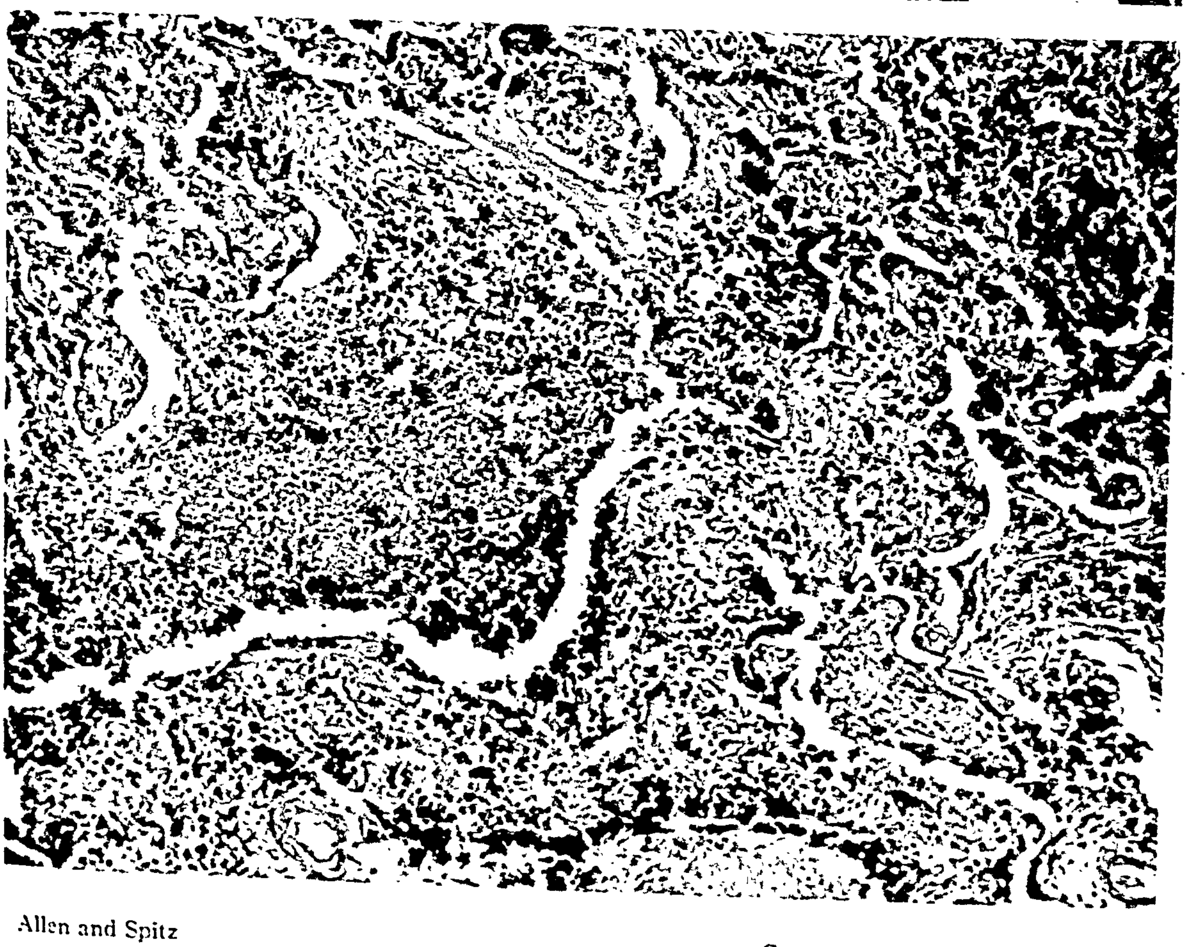

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Comparative Study of Scrub Typhus

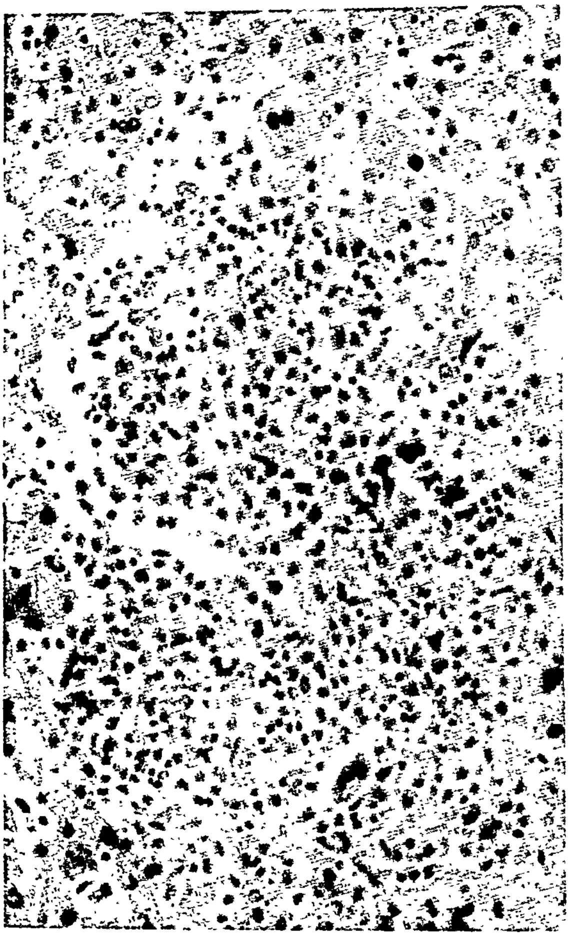
Plate 107

Fig. 37. Acc. 111021. Focal granulomatous inflammation near the midzone of a lobule of liver in a case of scrub typhus. Hematoxylin and eosin stain. $\times$ 230. Neg. 83011.

Fig. 38. Acc. 111021. Focal inflammation of liver in scrub typhus. Hepatic cells in center of focus have been lysed. Hematoxylin and eosin stain. $\times$ 450. Neg. 83012.

Fig. 39. Acc. 112241. Focal, midzonal granulomatous inflammation of liver in case of scrub typhus, showing evidence of phagocytosis by Kupffer cells. Hematoxylin and eosin stain. $\times$ 230. Neg. 83014.

Fig. 40. Acc. 105720-3A. Sickling of red blood cells within tributary of portal vein in liver from a case of epidemic typhus in an Egyptian. Hematoxylin and eosin stain. $\times$ 160. Neg. 83193.



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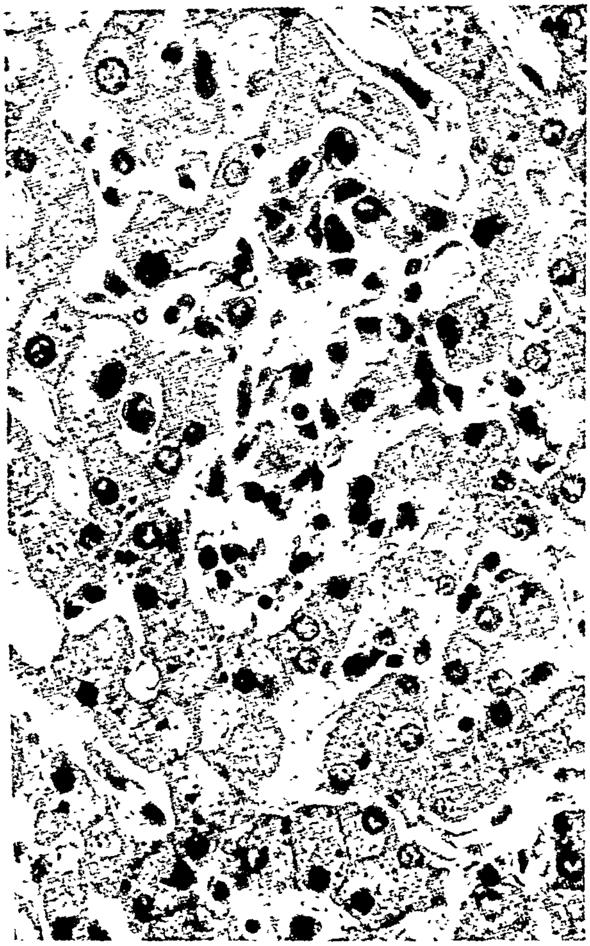

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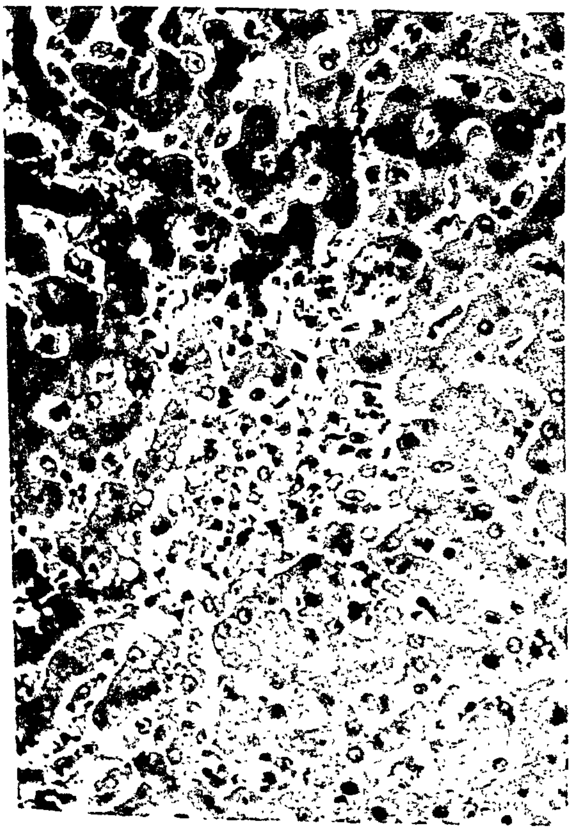

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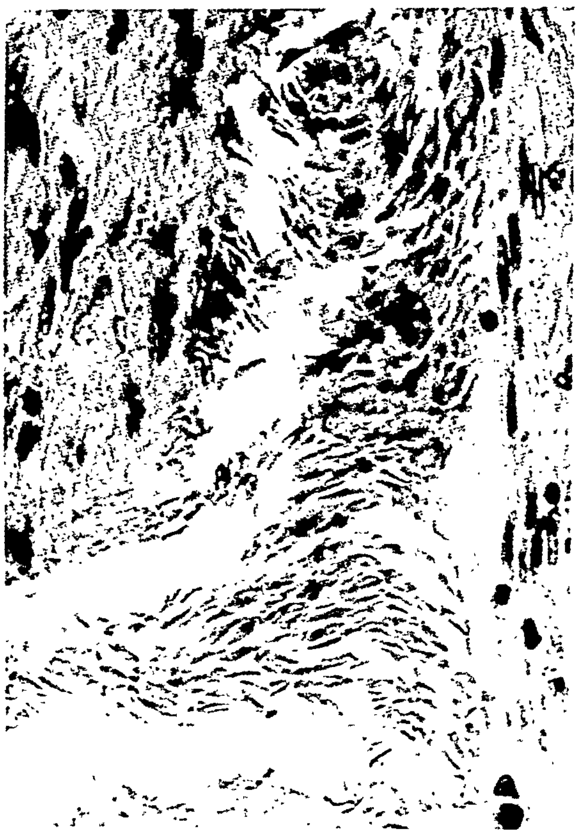

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Allen and Spitz

Comparative Study of Scrub Typhus

PLATE 108

FIG. 41. Acc. 112449. Interstitial pancreatitis in scrub typhus. The infiltrate, as in other organs, consists of various mononuclear cells. Hematoxylin and eosin stain. × 145. Neg. 82894.

FIG. 42. Acc. 105720-16A. Necrosis of spleen in epidemic typhus. An identical change occurs in scrub typhus. Hematoxylin and eosin stain. × 145. Neg. 82926.

FIG. 43. Acc. 94653. Section of spleen from a case of scrub typhus illustrating the large basophilic macrophage ("acute splenic tumor cell") characteristic of the infiltrate in all organs. Hematoxylin and eosin stain. × 515. Neg. 77035.

FIG. 44. Acc. 111024. Smear of spleen from case of scrub typhus showing rickettsiae in the cytoplasm of large mononuclear cells. Giemsa's stain, Wolbach's modification. × 1360. Neg. 83449.

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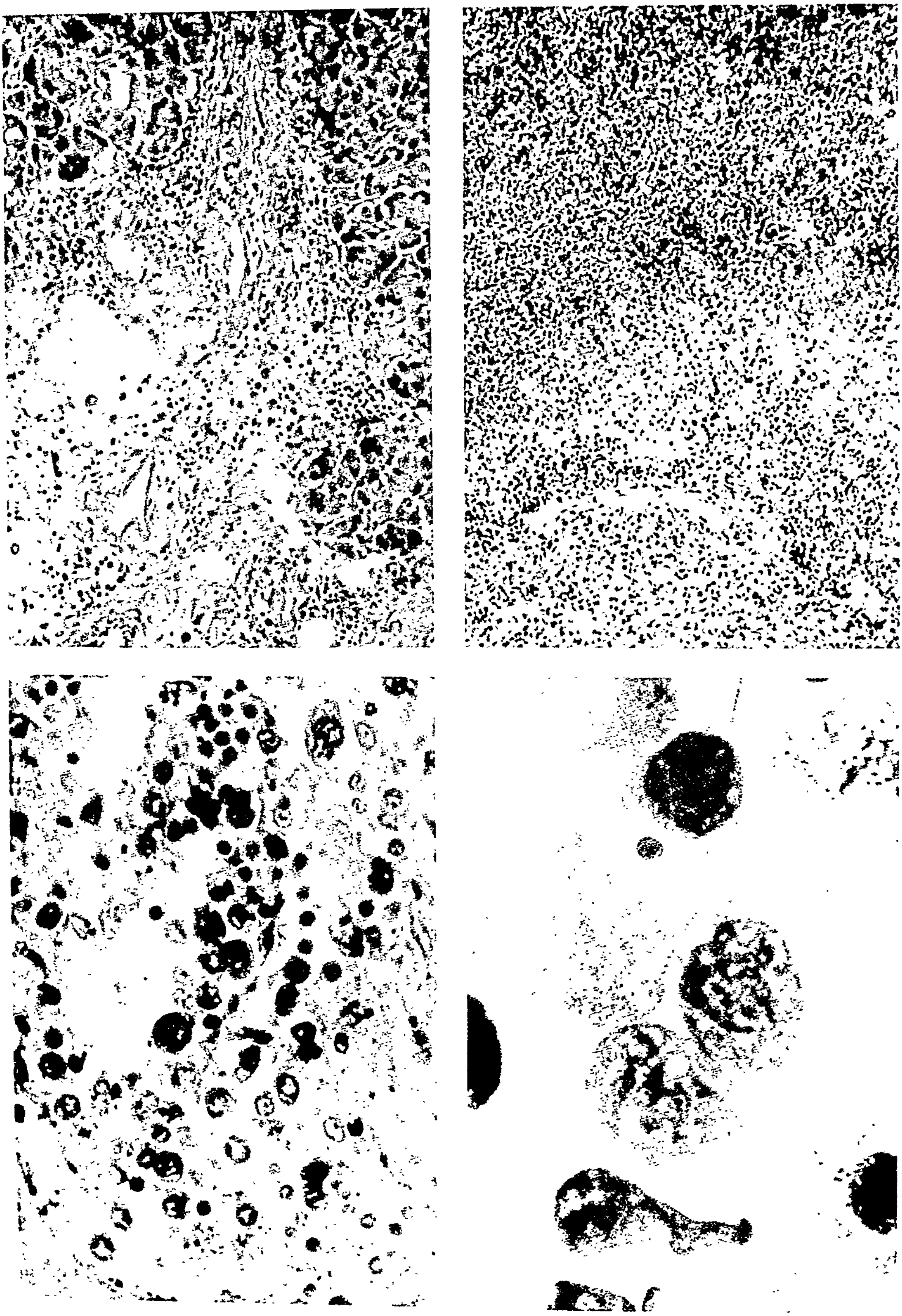

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Comparative Study of Scrub Typhus

PLATE 109

FIG. 45. Acc. 112247. Lymph node from a case of scrub typhus showing dilatation of sinuses, principally with acidophilic macrophages, many of which are vacuolated ("blister" histiocytes) and contain cellular débris. "Sinus catarrh." Hematoxylin and eosin stain. × 120. Neg. 83145.

FIG. 46. Acc. 111023. Lymph node from a case of scrub typhus showing early necrobiotic changes beginning in a germinal center. Hematoxylin and eosin stain. × 145. Neg. 82890.

FIG. 47. Acc. 111023. Lymph node of scrub typhus with more advanced necrobiosis than that shown in Figure 46. Hematoxylin and eosin stain. × 120. Neg. 83901.

FIG. 48. Acc. 101690. Lymph node of scrub typhus showing extension of the inflammation through the capsule. Hematoxylin and eosin stain. × 230. Neg. 78155.

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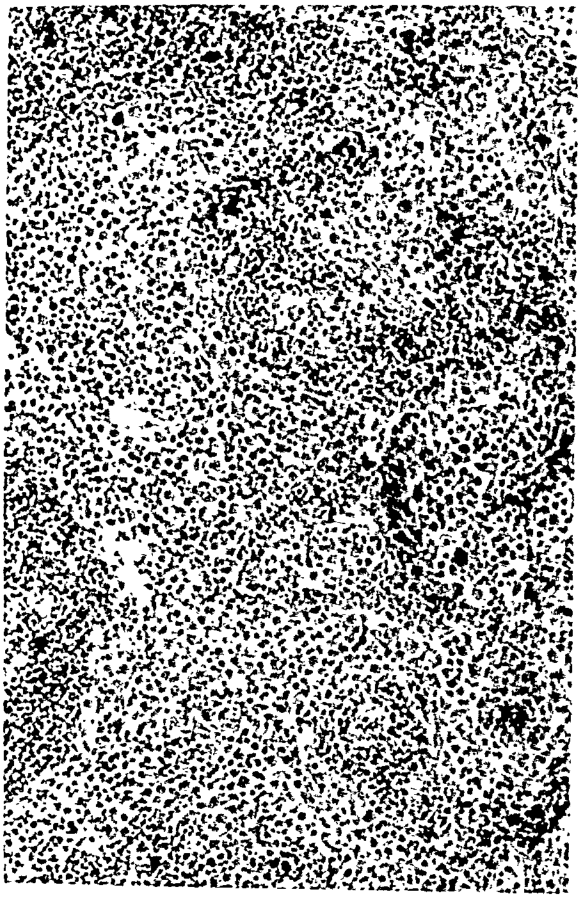

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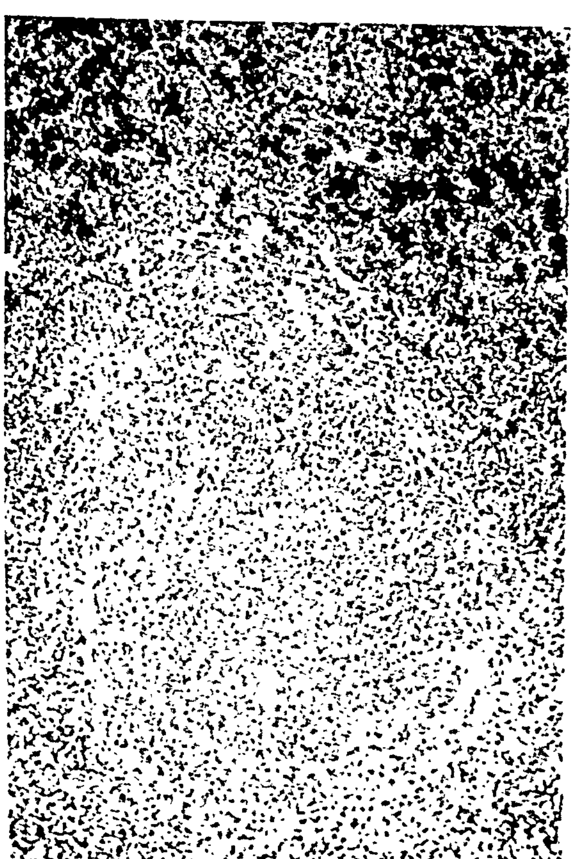

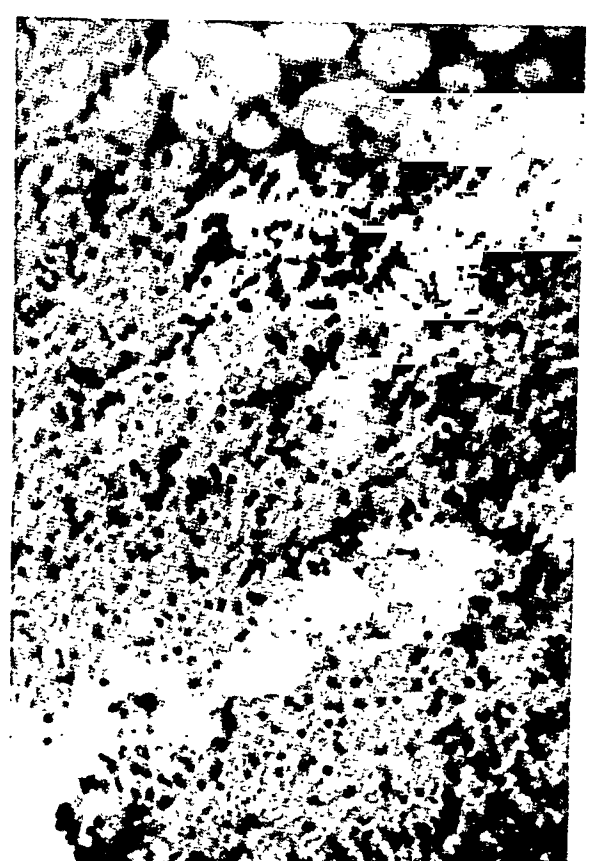

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Comparative Study of Scrub Typhus

PLATE 110

FIG. 49. Acc. 112232. Adrenal gland from a case of scrub typhus showing marked "tubular degeneration" (Rich) of cortex. Hematoxylin and eosin stain. × 175. Neg. 82923.

FIG. 50. Acc. 112449. Adrenal gland from a case of scrub typhus showing abundant fat in midfascicular zone, rather than depletion. Hematoxylin and eosin stain. × 120. Neg. 83142.

FIG. 51. Acc. 105720-22A. Thrombo-arteritis of adventitial arteries of adrenal in a case of epidemic typhus. Hematoxylin and eosin stain. × 145. Neg. 80315.

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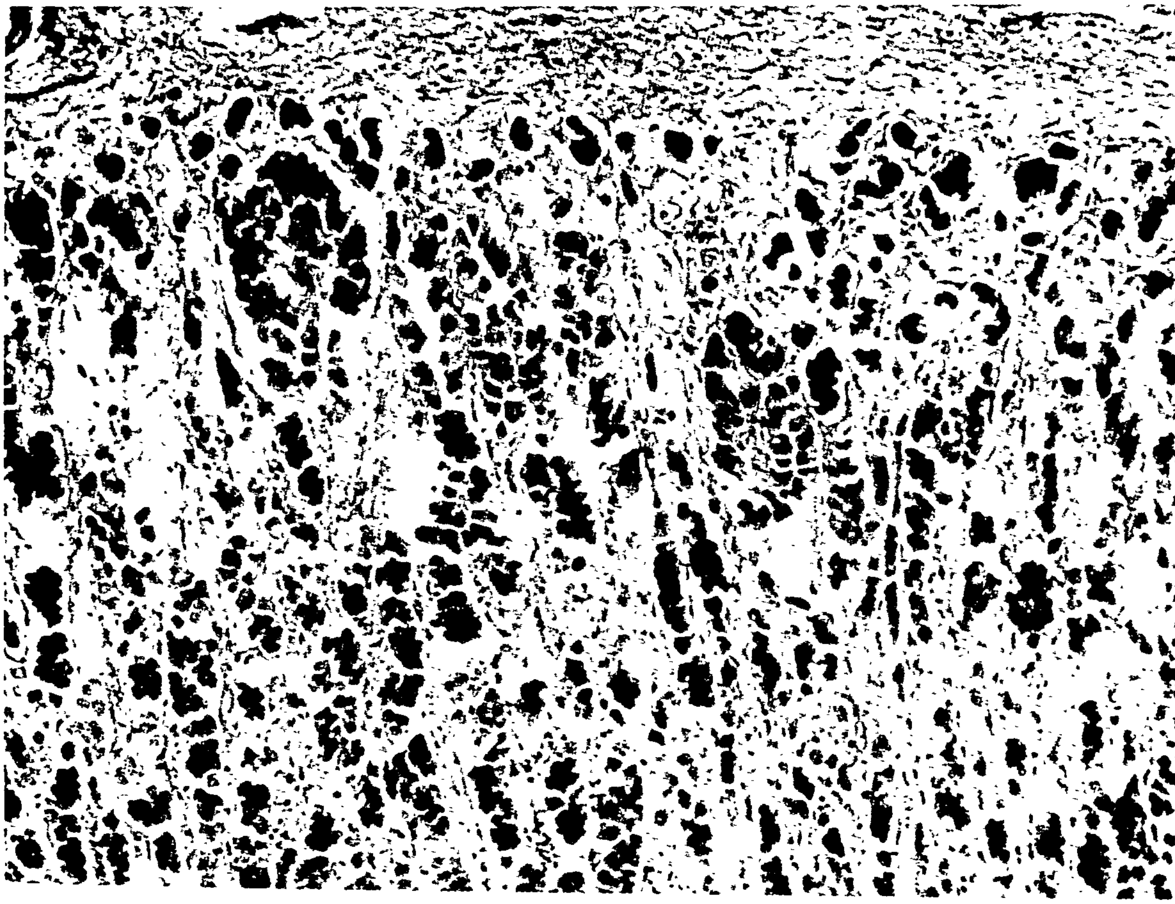

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PLATE 111

FIG. 52. Acc. 106767. Acute diffuse glomerulonephritis in a case of scrub typhus, showing particularly the absolute ischemia, the endothelial hyperplasia, and the fusion of capillary loops. Hematoxylin and eosin stain. × 255. Neg. 82917.

FIG. 53. Acc. 115681. Acute diffuse glomerulonephritis in a case of scrub typhus. Hematoxylin and eosin stain. × 500. Neg. 82555.

FIG. 54. Acc. 115682. Acute diffuse glomerulonephritis in a case of scrub typhus. Hematoxylin and eosin stain. × 255. Neg. 82903.

FIG. 55. Acc. 105720-20A. Acute diffuse glomerulonephritis in a case of epidemic typhus. Hematoxylin and eosin stain. × 350. Neg. 82559.

FIG. 56. Acc. 75608. Acute diffuse glomerulonephritis in a case of Rocky Mountain spotted fever. Hematoxylin and eosin stain. × 330. Neg. 83157.

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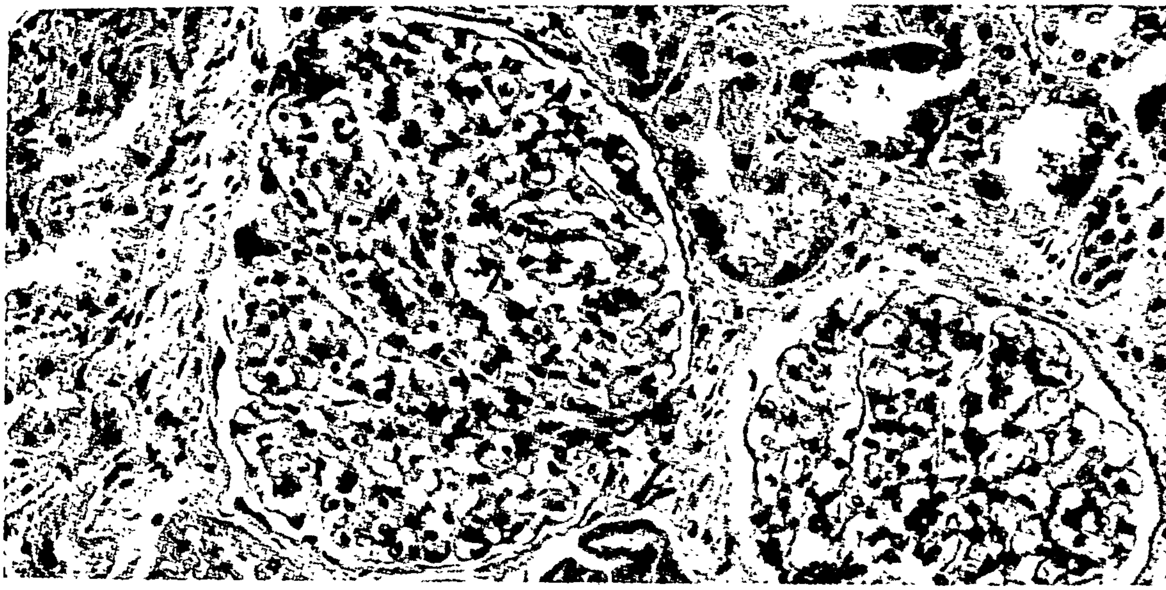

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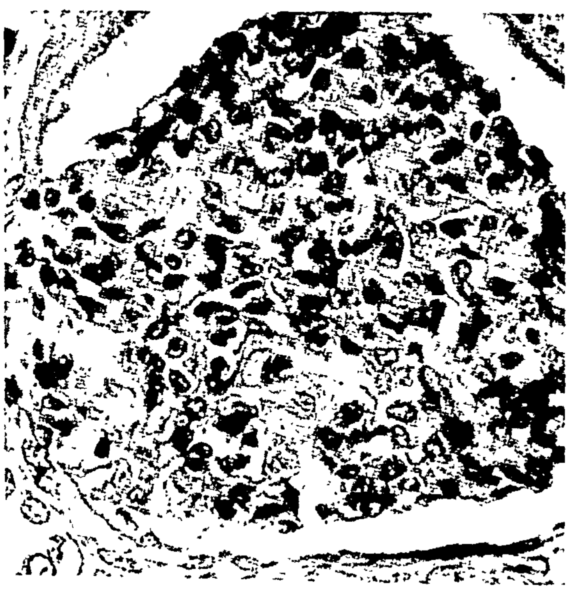

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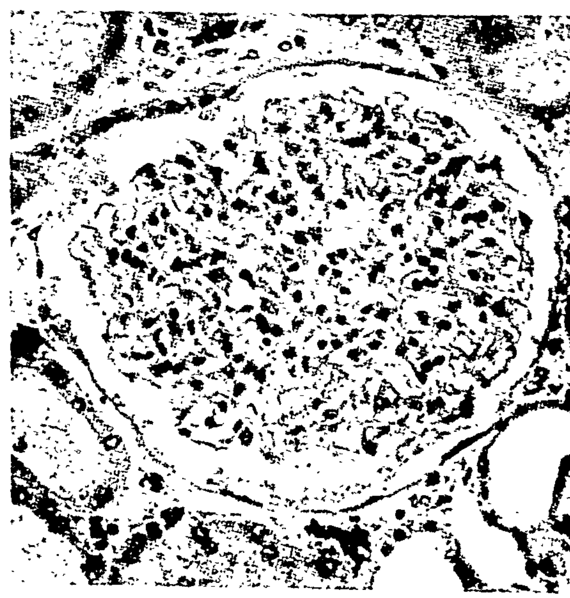

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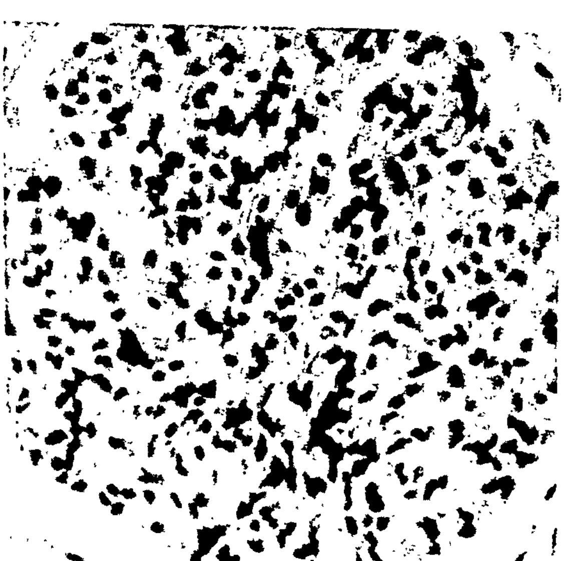

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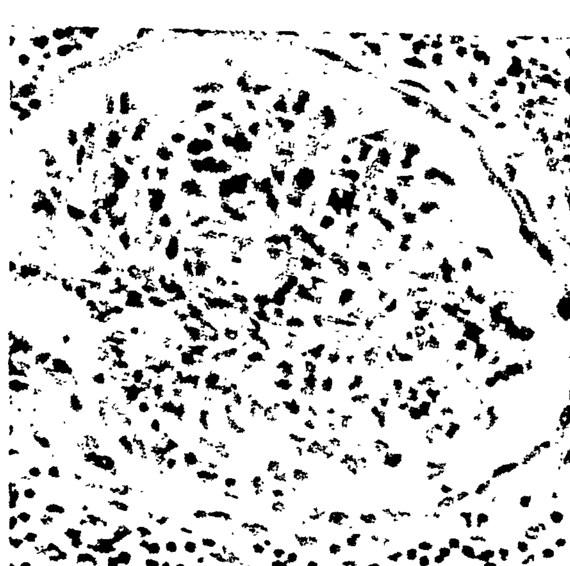

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Comparative Study of Scrub Typhus

PLATE 112

FIG. 57. Acc. 94537. Section of medulla of kidney in a case of scrub typhus, showing a striking concentration of mononuclear cells within the lumen of a peritubular vein. The cells include lymphocytes, plasma cells, possibly Türk cells, basophilic and acidophilic macrophages, the latter manifesting evidence of cytophagocytosis. Hematoxylin and eosin stain. × 500. Neg. 82550.

FIG. 58. Acc. 94655. Vacuolization and hyaline droplet formation within the epithelium of proximal convoluted tubules in a case of scrub typhus with no glomerulonephritis. Hematoxylin and eosin stain. × 500. Neg. 82558.

FIG. 59. Acc. 111605. Degeneration and regeneration of epithelium of distal tubules of glomerulonephritic kidney from a case of scrub typhus. A mitotic figure may be noted. Hematoxylin and eosin stain. × 500. Neg. 82560.

FIG. 60. Acc. 112262. Acute diffuse interstitial nephritis in a case of scrub typhus. Hematoxylin and eosin stain. × 145. Neg. 82554.

FIG. 61. Acc. 111605. Phlebitis of interlobar vein in association with focal interstitial nephritis in a case of scrub typhus. Hematoxylin and eosin stain. × 120. Neg. 82551.

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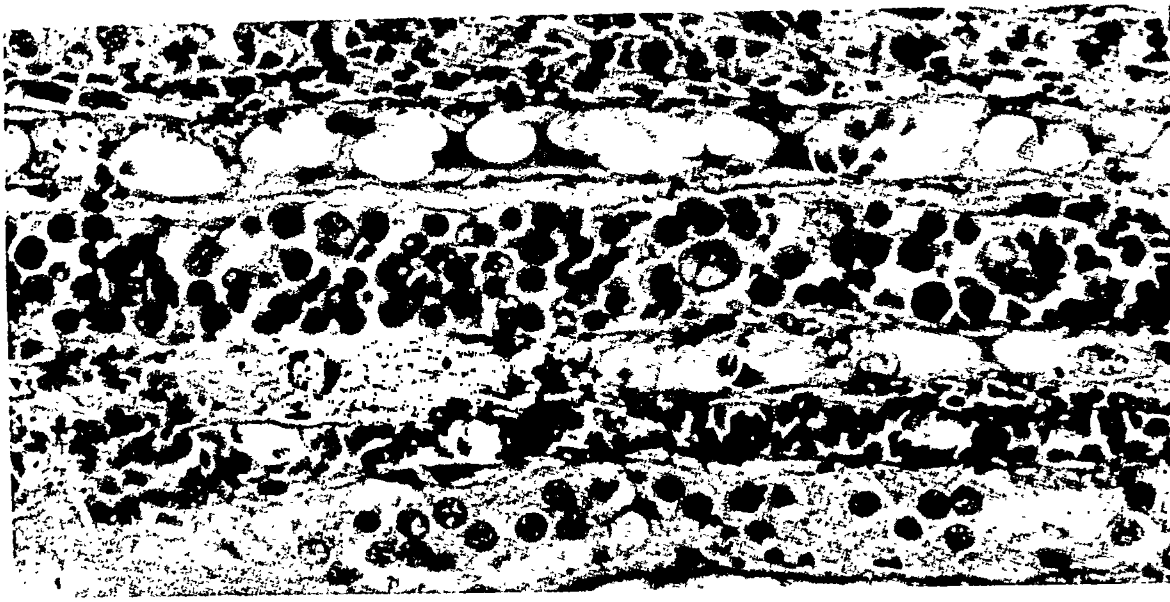

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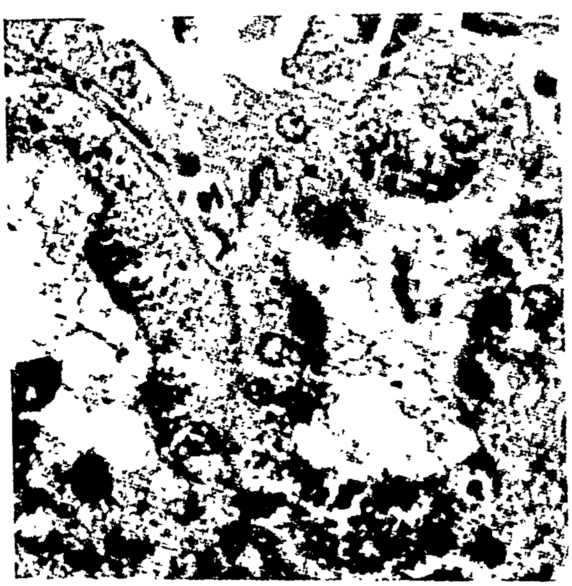

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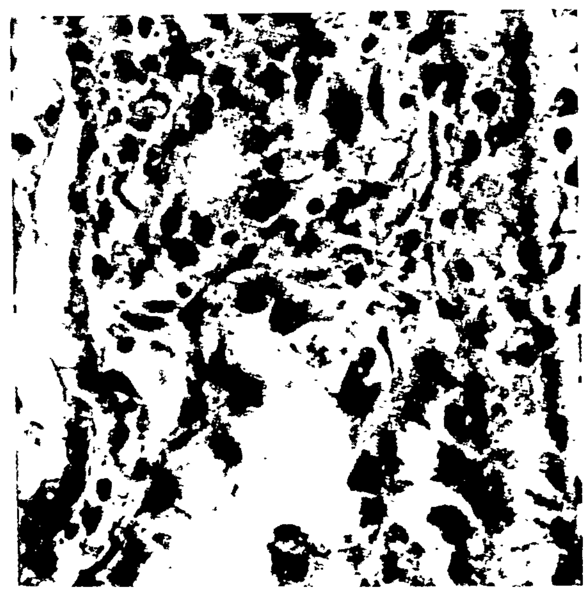

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PLATE 113

FIG. 62. Acc. 112247. Interstitial orchitis and thrombophlebitis in a case of scrub typhus. Hematoxylin and eosin stain. × 230. Neg. 82919.

FIG. 63. Acc. 112449. Tubular atrophy, and interstitial edema and inflammation in a case of scrub typhus. Hematoxylin and eosin stain. × 145. Neg. 82915.

FIG. 64. Acc. 112240. Fibrinoid degeneration of a segment of a testicular vein in a case of scrub typhus. There is evidence of atrophy even in the absence of appreciable interstitial inflammation. Hematoxylin and eosin stain. × 230. Neg. 82717.

FIG. 65. Acc. 105720-16A. Thrombo-arteritis in rete testis in a case of epidemic typhus. Hematoxylin and eosin stain. × 145. Neg. 82909.

FIG. 66. Acc. 105720-21A. Thrombo-arteritis with arterionecrosis, and periarterial mononuclear response in the tongue in a case of epidemic typhus. Hematoxylin and eosin stain. × 330. Neg. 80317.

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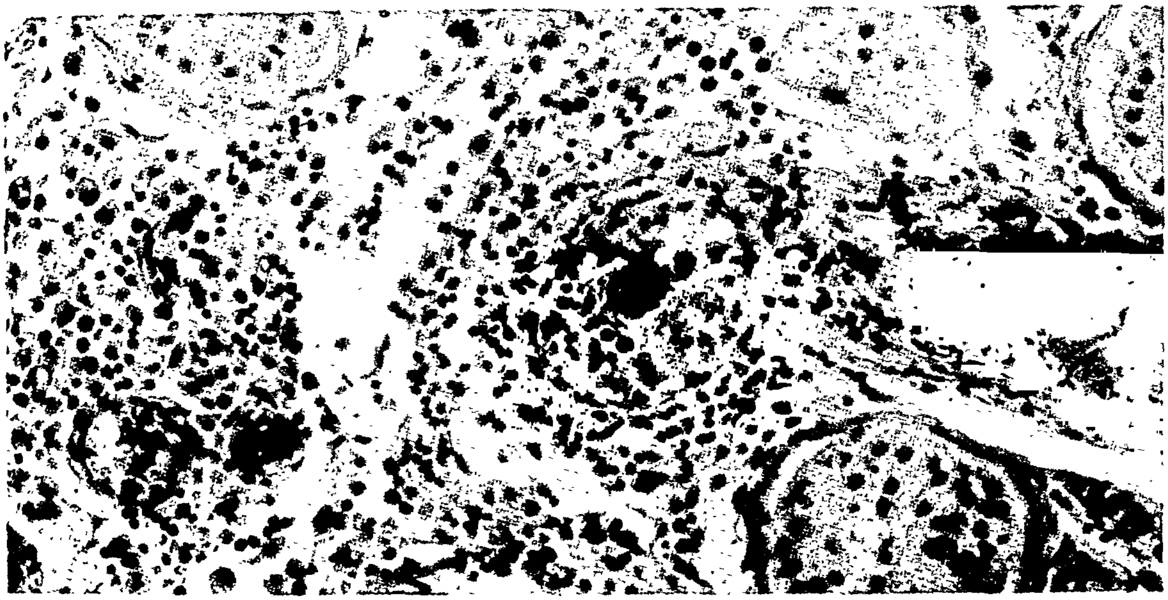

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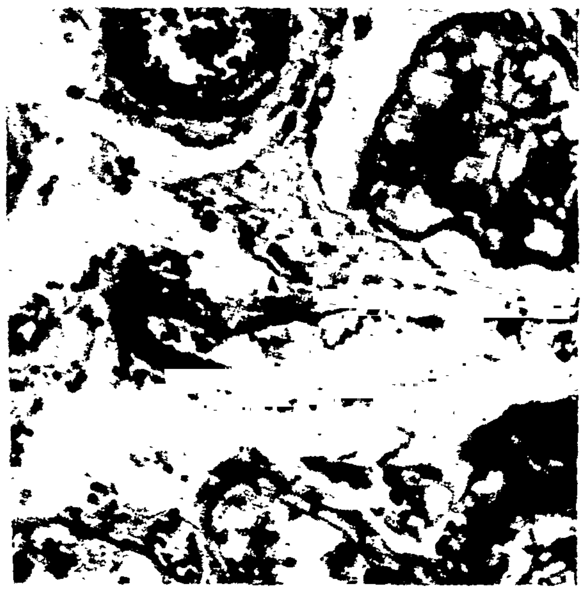

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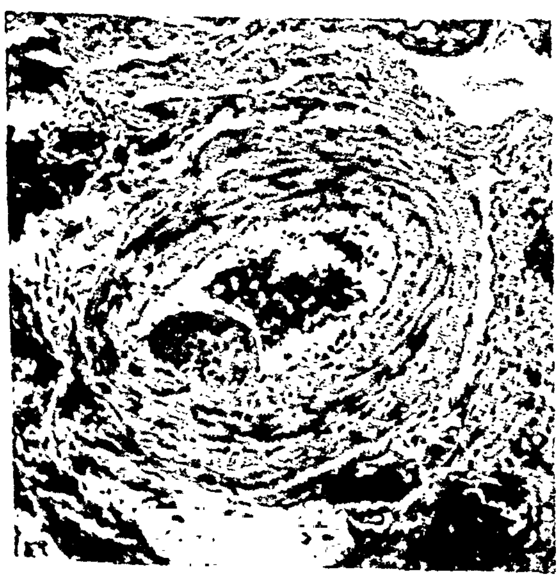

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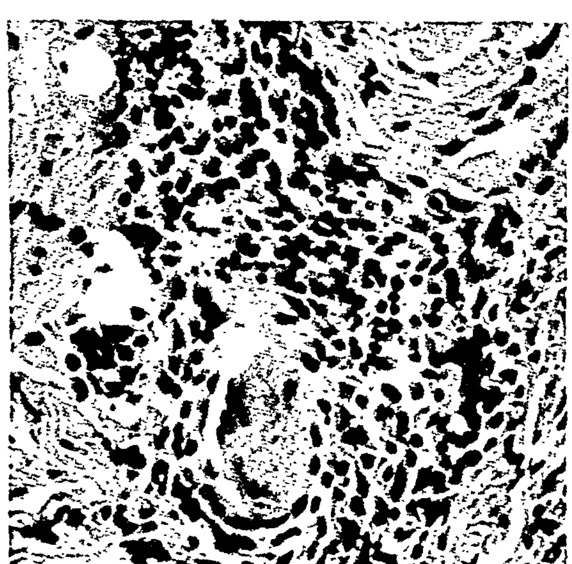


PLATE 114

FIG. 67. Acc. 94537. Nodule in brain from a case of scrub typhus. Hematoxylin and eosin stain. × 450. Neg. 82939.

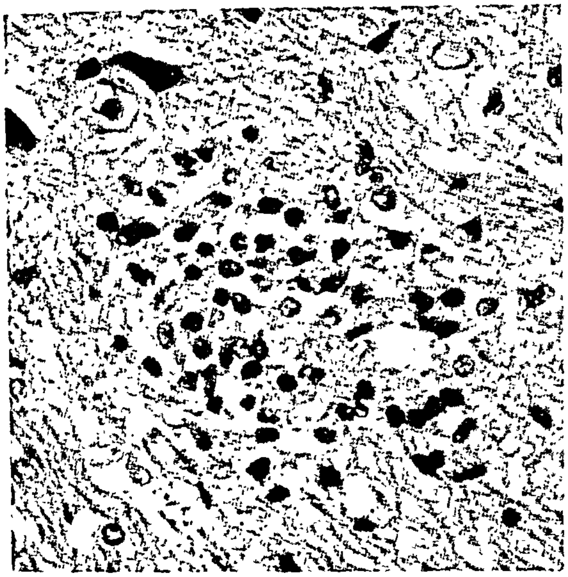
FIG. 68. Acc. 105720-13A. Nodule in brain from a case of epidemic typhus. under same magnification as Figure 67. The larger size and somewhat greater diversity of types of cells may be noted. Hematoxylin and eosin stain. × 450. Neg. 82929.

FIG. 69. Acc. 111023. Nodule of brain in scrub typhus showing the disruption of the argyrophilic, capillary wall. Wilder's silver stain. × 810. Neg. 82504.

FIG. 70. Acc. 105720-21A. Nodule of brain from a case of epidemic typhus showing the argyrophilic capillary wall. Wilder's stain. × 810. Neg. 82506.

FIG. 71. Acc. 79689. "Nodule" of brain from a case of Rocky Mountain spotted fever, showing a collection of cells about a prominent thrombosed arteriole. Hematoxylin and eosin stain. × 400. Neg. 82899.

FIG. 72. Acc. 79689. Early microinfarct of brain in Rocky Mountain spotted fever, with a central thrombosed arteriole and characteristically disrupted parenchyma. Hematoxylin and eosin stain. × 230. Neg. 83007.



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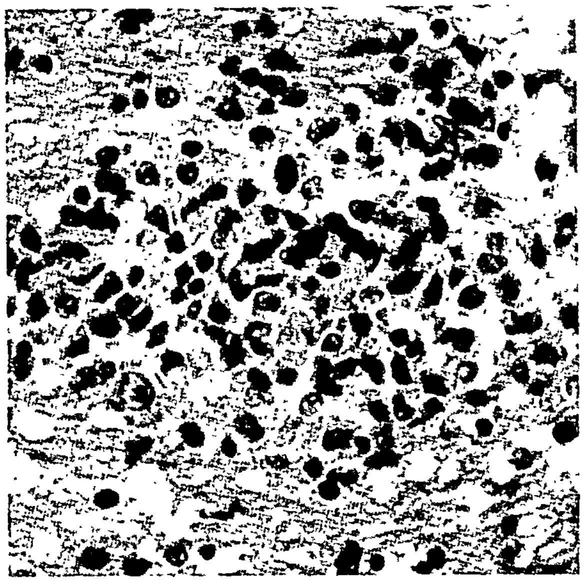

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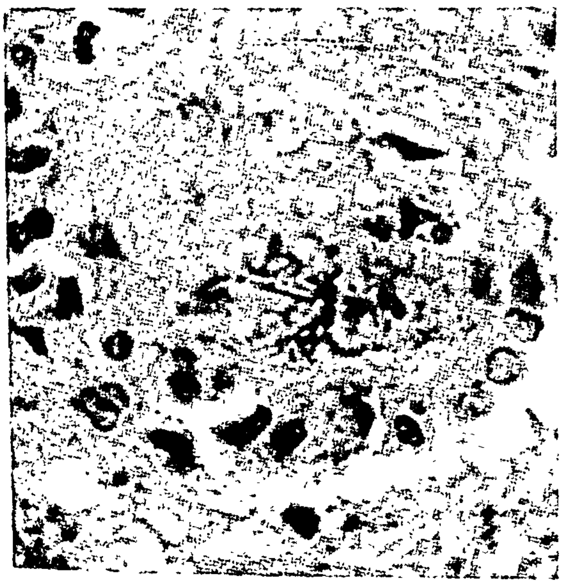

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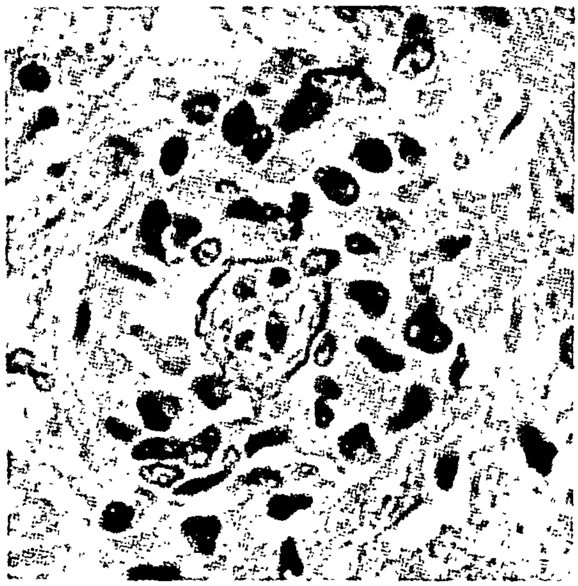

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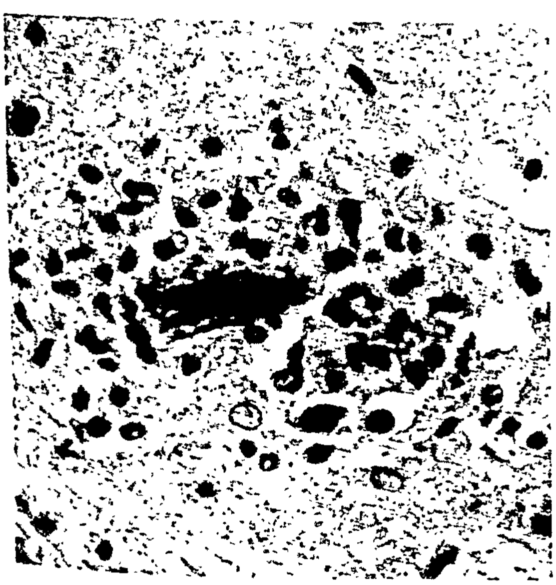

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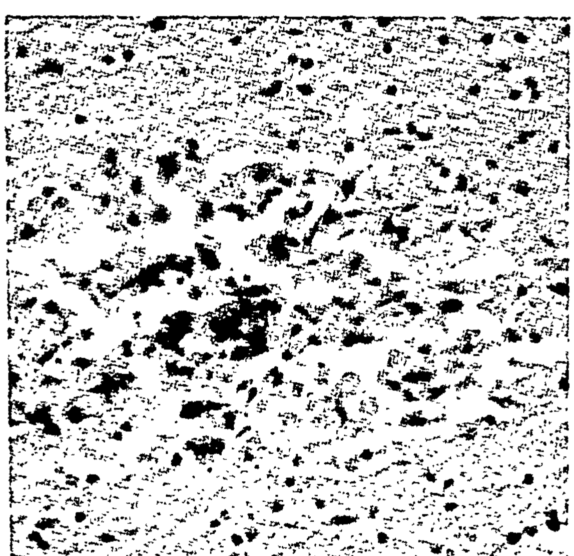

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PLATE 115

FIG. 73. Acc. 112435. Marked leptomeningeal reaction in a case of scrub typhus. Hematoxylin and eosin stain. × 110. Neg. 83156.

FIG. 74. Acc. 112435. Focal hemorrhages in the supra-optic nucleus in a case of scrub typhus. Hematoxylin and eosin stain. × 145. Neg. 82930.

FIG. 75. Acc. 111020. Satellitosis, in cerebral cortex of a case of scrub typhus. Hematoxylin and eosin stain. × 500. Neg. 83005.

FIG. 76. Acc. 105720-22A. Thrombo-arteriolitis and perivascular mononuclear cell infiltration of posterior lobe of pituitary gland in a case of epidemic typhus. Hematoxylin and eosin stain. × 230. Neg. 82925.

FIG. 77. Acc. 101508. Malarial "granuloma." There is a rosette of glial cells and a rim of demyelinization. Hemorrhage is absent in this instance. The parasites are visible in the capillaries as black dots. The malarial lesions tend to stimulate the microinfarcts of Rocky Mountain spotted fever more than they do the nodules of scrub or epidemic typhus. Hematoxylin and eosin stain. × 280. Neg. 77498.

FIG. 78. Acc. 41410. A "nodule" in the brain from a case of Chagas' disease. This lesion tends to follow the pattern of scrub typhus or epidemic typhus. Hematoxylin and eosin stain. × 450. Neg. 83009.

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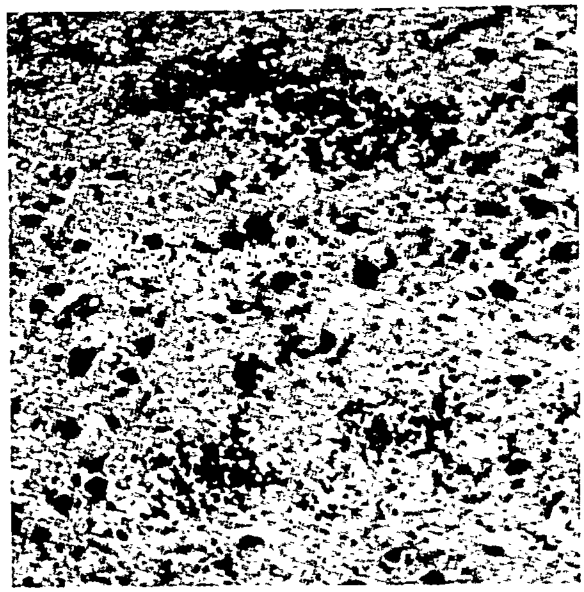

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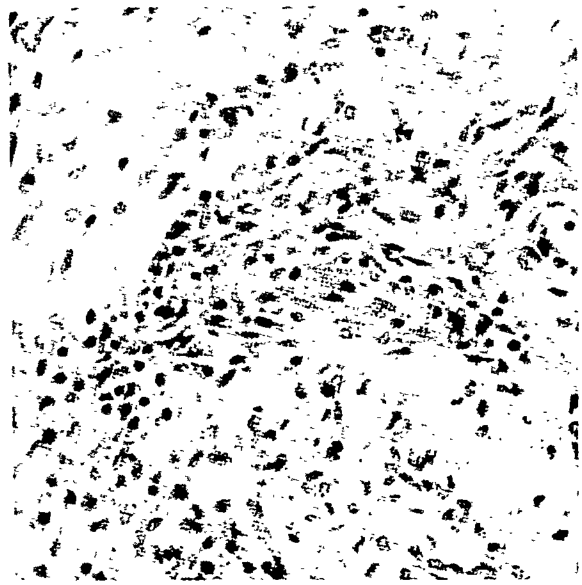

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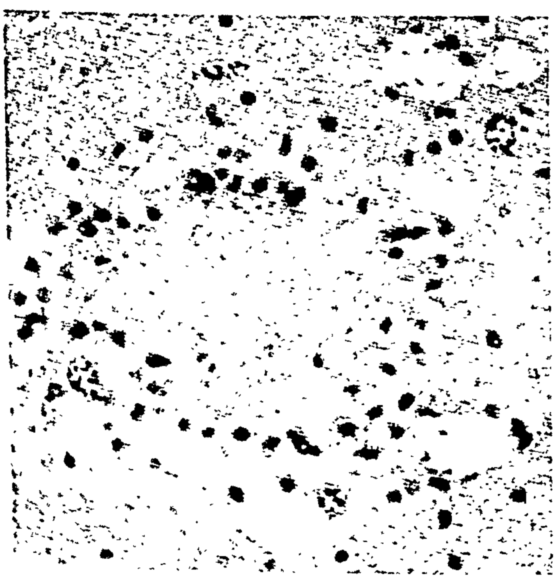

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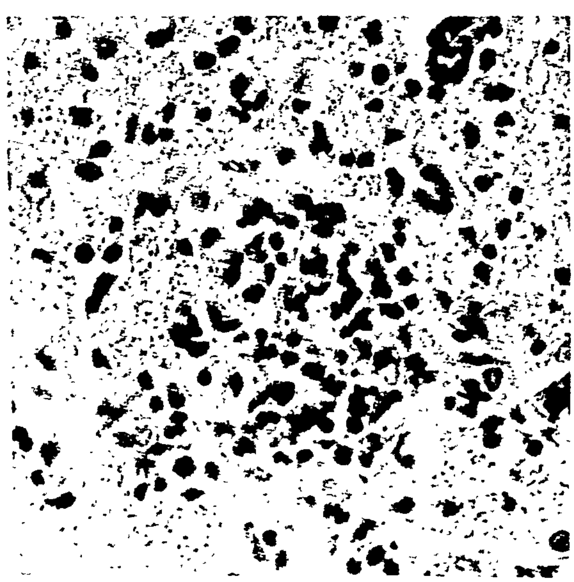

Plate 116

Fig. 79. Acc. 111026. A section of testis from a case of scrub typhus showing a mononuclear cell infiltration of the wall of an artery as if by extension from the interstitial infiltrate. Hematoxylin and eosin stain. × 145. Neg. 82893.

Fig. 80. Acc. 112247. Thrombophlebitis within the testis in a case of scrub typhus. The accompanying artery is spared. Hematoxylin and eosin stain. × 145. Neg. 82891.

Fig. 81. Acc. 94722. Pulmonary artery from a case of scrub typhus showing the type of subendothelial vacuolization and intimal edema that is associated with allergic manifestations, as in bronchial asthma. Interstitial pneumonitis was present in this instance. Hematoxylin and eosin stain. × 145. Neg. 83194.

Fig. 82. Acc. 105720-18A. A cutaneous artery from a case of epidemic typhus showing either a thrombo-arteritis or a true, intimal verruca. The uniform granularity of the hillock and the underlying arterial wall, and the lifting of the endothelium are some of the reasons for considering the vasculogenesis of the intimal mound. The degeneration involves the media and adventitia as well as intima. Hematoxylin and eosin stain. × 230. Neg. 82896.

Fig. 83. Acc. 105720-10A. Acute diffuse glomerulonephritis in a case of epidemic typhus. This section illustrates another site of vascular lesions. There are swelling, hyperplasia and hyperchromasia of the endothelial cells of the glomerular capillaries as well as focal karyorrhexis and fibrinoid degeneration. Hematoxylin and eosin stain. × 255. Neg. 82902.

Fig. 84. Acc. 105720-20A. Sickling of red blood cells within an interlobular vein in a case of epidemic typhus in an Egyptian native. Hematoxylin and eosin stain. × 330. Neg. 83198.

Fig. 85. Acc. 94875. Necrotizing arteritis in the midst of a focus of interstitial nephritis in a case of Rocky Mountain spotted fever. Hematoxylin and eosin stain. × 230. Neg. 82906.

Fig. 86. Acc. 94423. Necrotizing arteritis of the scrotum from a case of Rocky Mountain spotted fever. ×255. Neg. 82903.

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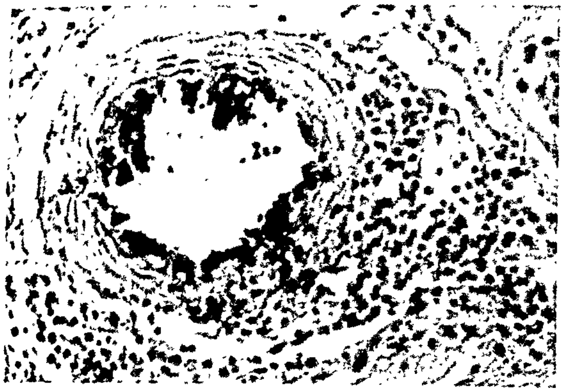

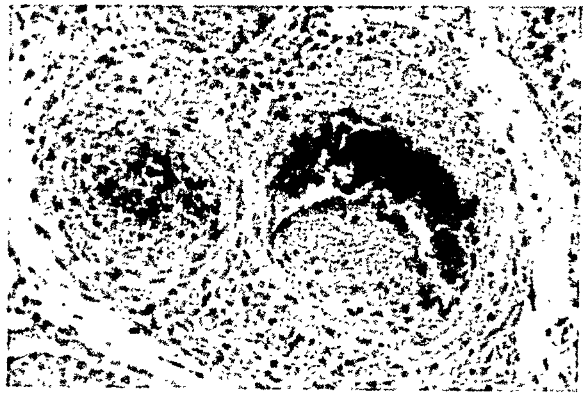

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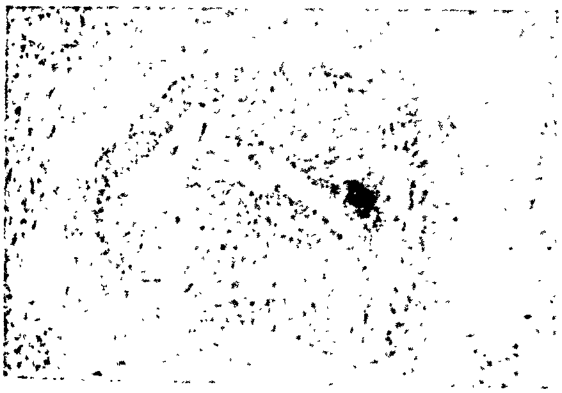

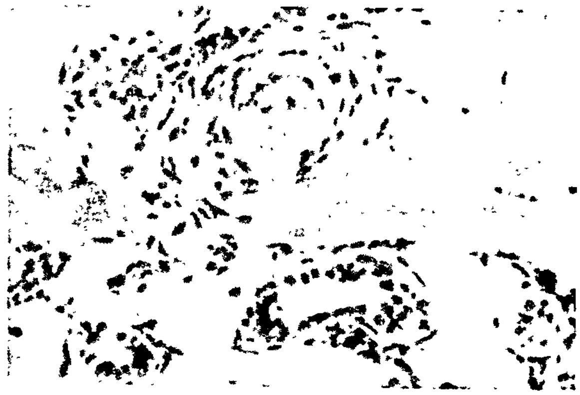

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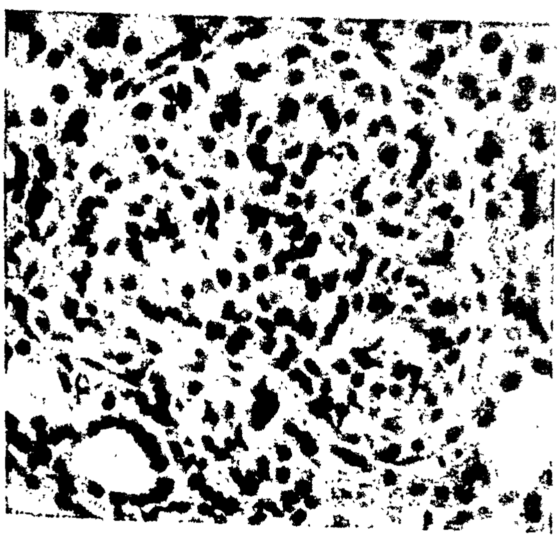

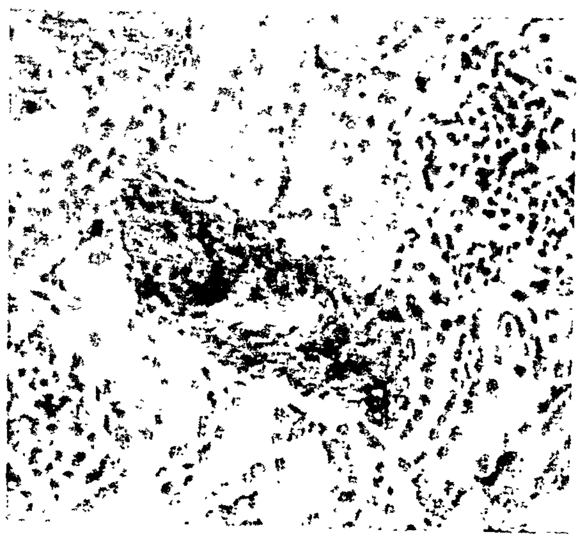

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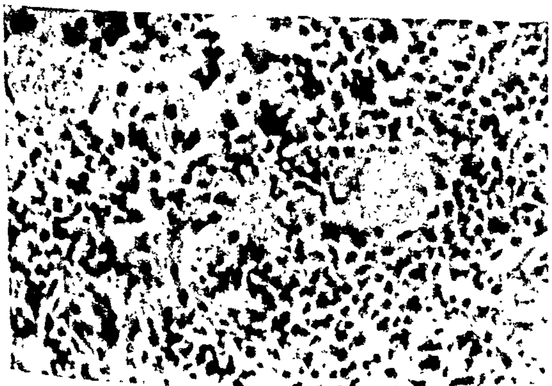

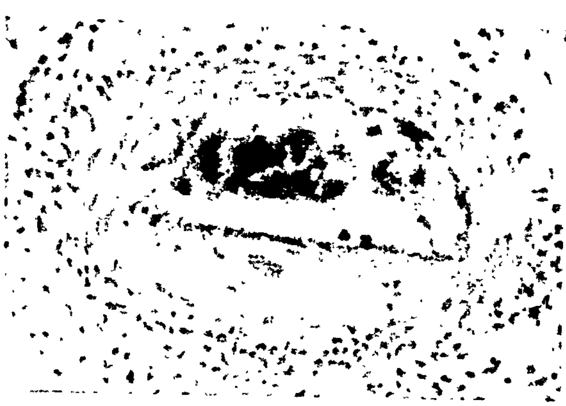

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Allen and Spitz

Comparative Study of Scrub Typhus

MALIGNANT LYMPHOMA (SO-CALLED LEUKEMIA) IN DOGS *

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From the point of view of comparative pathology, the leukemic and leukemia-like diseases of the lower animals have engaged the attention of many investigators. Such studies have largely been directed toward a better understanding of similar diseases as they occur in man. The problem of leukemia in man finds its counterpart in the leukemia affecting animals.

With few exceptions, however, the disease in dogs has been only superficially examined, although it is fair to state that considerable information is at hand concerning the lymphomas as they affect this species. In many instances, hematologic and histologic studies were not performed and frequently the results of such examinations have been erroneously interpreted. The bewildering nomenclature of the human disease has often been applied to the canine disease without due consideration for the hematologic and pathologic alterations as they actually exist. While both the human and canine disease have many features in common, distinct differences occur and many of the classifications proposed for the former do not logically apply to the latter.

In this study, therefore, we propose to classify and describe the hematologic and pathologic aspects of the lymphomas in dogs, together with brief notes on the clinical features.

Literature

The literature on the canine leukemias is very voluminous and many papers have been omitted from our bibliography due to lack of adequate pathologic and hematologic data.

Although Leisering [1] (1858) first described leukemia in the domesticated animals (in a horse), Siedamgrotzky [2] (1871) first reported lymphatic leukemia in a dog in which the lymph nodes and spleen were enlarged and the ratio of white to red cells was 1 to 15. Bollinger [3] (1874) designated as splenic and lymphatic leukemia a condition in a dog that showed hyperplasia of the spleen and lymph nodes, leukemic infiltrations of the liver and lungs, and a ratio of white to red cells after death of 1 to 5. Shortly after, Cadiot [4] (1892), Stockmann [5] (1893), Smythe [6] (1898), Olt [7] (1899), and others described cases of leukemia, principally of the lymphatic type, in dogs.

Weil and Clerc [8] (1904) were among the first to do blood examinations and noted lymphatic leukemia and aleukemic lymphatic leukemia

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in 2 dogs. In the first dog the white cells were 320,000: lymphocytes, 88 per cent; monocytes, 3 per cent; plasma cells, 1 per cent; polynuclear leukocytes, 8 per cent. The lymph nodes, liver, kidneys, spleen, mammary gland, and bone marrow showed lymphocytic infiltrations. The exact nature of the disease is unknown in the second case as these blood changes were absent and no autopsy was performed. In 1905, the same authors [9] reported 2 cases of myelogenous leukemia. A leukocytosis existed in both dogs: in one there were 165,000 white cells with 93 per cent polynuclear leukocytes and 7 per cent mononuclear leukocytes, and in the other the white cells totaled 50,000 with 88 per cent polynuclear leukocytes, 0.4 per cent eosinophils, 5.6 per cent monocytes, 4 per cent myeloblasts, and 2 per cent myelocytes. The bone marrow and other organs were thought to be infiltrated with myeloblasts. Opie [10] (1928) stated that the blood changes in these 2 cases do not establish the diagnosis of leukemia. Additional examples of leukemia in dogs, the majority of them being of the lymphogenous type, were noted by Cadiot and Weil [11] (1904), Lellmann [12] (1904), Jakob [13] (1907), Feuereissen [14] (1907), Jäger [15] (1907), and Ball [16] (1912). Lüdke [17] (1910) designated a condition in a dog as myelogenous leukemia from the blood and tissue changes. According to Wirth [18] (1931), the blood findings in the cases of Olt,[7] Jakob,[13] and Abendroth [19] (1913) could be termed myelogenous and considered as an expression of myelosis. Dahlström and Henschen [20] (1918) collected 50 cases of leukemia in dogs from the literature and concluded that the disease is not particularly frequent. Crocker[21] (1919) cited 1 case of leukemia from autopsy examinations of 1548 dogs. Milks [22] (1919) noted pseudoleukemia in a dog in which the leukocytes were normal, the spleen and lymph nodes were greatly enlarged, and these organs and the liver were infiltrated with lymphocytes. Spaulding [23] (1920) mentioned 6 cases of leukemia and 7 of pseudoleukemia in a group of about 15,000 dogs. In 1 animal the white cells were increased and the differential was: polynuclear leukocytes, 49 per cent; mononuclear leukocytes, 11 per cent; lymphocytes, 3 per cent; and eosinophils, 37 per cent. Burnett [24] (1920) properly criticized the criteria by which the diagnosis of leukemia was made by Spaulding since hematologic data were not characteristic. Burnett further stated that myelogenous leukemia has not been reported in the domesticated animals.

Wirth [25] (1920) described 2 cases of lymphatic leukemia and 11 of myelogenous leukemia in dogs. In the former, in 1 animal the leukocytes were 24,000 with 60.1 per cent polynuclear leukocytes and 39.9 per cent lymphocytes; and in the latter the white cells were twice the normal number, with 43.2 per cent lymphocytes. Opie [10] considered

these cases as examples of aleukemic lymphoma rather than leukemia. In the 11 dogs, Wirth stated that although the typical blood pictures of myelogenous leukemia as they occur in man were absent, the histologic data were suggestive of myelogenous leukemia or subleukemia, as judged by the myeloid cells in the bone marrow and other organs. Lund [26] (1927) found 2 cases of myelosis in 6 dogs and Julliard [27] (1928) believed that myeloid leukemia is predominant in this species. Opie [10] critically reviewed the literature concerning leukemia in animals and concluded that in dogs, lymphatic leukemia occasionally occurs and that lymphomatous tumors are not uncommon. The nature of the disease described as myelogenous leukemia is as yet uncertain.

Knuth,[28] (1929) in his review of animal leukemias, quoted Weil and Clerc [9] and Wirth [25] to indicate that although the microscopic immature myeloid infiltrations are typical of myelosis, the blood picture is that of a leukocytosis. Milks and Olafson [29] (1929) described pseudoleukemia in a dog with 12,000 white cells and 77 per cent polynuclear leukocytes, 19 per cent monocytes, and 4 per cent lymphocytes. The lymph nodes, spleen, liver, and pancreas were enlarged and infiltrated with lymphocytic cells. Fourie and Ziehn [30] (1930) reported lymphoid aleukemia in a dog; *viz.*, a generalized lymphosarcomatosis, with 3000 white cells and a differential of 77 per cent lymphocytes, 23 per cent polynuclear leukocytes and monocytes. Wirth [18] (1931) stated that in the dog the myelosis constitutes approximately 80 per cent and the lymphadenosis comprises between 10 and 20 per cent.

Concerning the etiologic factors, Share-Jones [31] (1927) and Jármai [32] (1933) observed the apparent development of lymphogenous leukemia in connection with trauma. The history sometimes suggested that the disease was brought about by an intercurrent condition such as pregnancy (Stockmann [5]), overexertion (Timm,[33] 1919), 5 days after mating (Smythe [6]), and dochmiasis (Henschen [34]).

Feldman [35] (1932) classified leukemia, pseudoleukemia, etc. as lymphoblastoma with lymphoma as the benign and lymphocytoma as the malignant type. He suggested the use of leukemic or aleukemic before lymphocytoma to designate the hematologic changes. Myelogenous leukemia was termed myeloblastoma and he referred to the studies of Weil and Clerc [9] and Wirth [25] as contributing to the knowledge of the hematology associated with this condition. Wirth and Baumann [36] (1933) reported lymphatic leukemia with typical blood pictures and further stated that all cases in dogs are lymphatic although the blood picture may be that of a leukocytosis, thus reversing Wirth's [18, 25] previous beliefs. Hutyra, Marek, and Manninger [37] (1938) described the leukemias in animals, the descriptions being largely based

on the studies of some of the previously mentioned investigators. Collins [38] (1939) reported an instance of leukemia with normal white cell and differential counts. The lymph nodes were enlarged and the kidneys, spleen, and liver grossly normal. Cherry [39] (1940) noted lymphatic leukemia with 26,050 white cells and macroscopic lymphadenopathy and normal spleen and liver. Atkinson [40] (1941) described a case of leukemia with 56,040 leukocytes and a differential count of 45 per cent segmented neutrophils, 6 per cent eosinophils, 43 per cent lymphocytes, and 6 per cent monocytes. The liver, spleen, and lymph nodes were enlarged. In the latter 3 cases, no microscopic reports were given and the diagnosis of leukemia is questionable. Probably the most accurate statement pertaining to the leukemias in dogs was that of Engelbreth-Holm [41] (1942), who considered that lymphogenous leukemia has somewhat peculiar features and that two different types of the disease occur: one form corresponds to that observed in other animals and in man; the other has the same typical visceral lesions but shows changes in the blood that are not typical of lymphogenous leukemia but rather resemble nonspecific leukocytosis. The latter form constitutes the majority of cases in this animal.

Material and Methods

The cases studied represent dogs brought to the small animal hospital of one of us (F. B.) for treatment. Blood examinations were performed by the usual methods. Bone marrow was aspirated from the crest of the ilium and the percentage distribution of marrow cells determined by the previously described morphologic criteria.[42, 43] Dry imprint preparations of lymph nodes secured for biopsy, and of lymph nodes and spleen made immediately after killing the animal with soluble pentobarbitol were stained with Wright's and May-Grünwald-Giemsa stains. In some instances, Isaac's method [44] (brilliant cresyl blue and Wright's) was employed to stain the blood, aspirated marrow, lymph node imprints, and smears of lymph nodes previously macerated in the serum of the same animal. Necropsies were performed immediately and the tissues were fixed in Zenker's formalin solution. The sections were stained with hematoxylin and eosin, Dominici's stain,[45] and Wilder's reticulum stain.[46]

Classification

A variety of terms such as lymphoblastoma, lymphoma, lymphosarcoma, leukemia, pseudoleukemia, lymphadenosis, lymphocytoma, lymphomatosis, and others have been applied to tumor-like enlarge-

ments of the lymph nodes in both human and veterinary medicine. We have followed the terminology of Gall and Mallory[47] in designating the diseases we encountered in dogs as malignant lymphoma since in our opinion it best expresses the anatomic-pathologic features present.

A study of the imprint and sectioned lymph nodes revealed four distinct categories into which the cases could be subdivided on the basis of the cytologic structure of the predominant cell type. These were as follows: (1) lymphoblastic type (6 cases); (2) lymphosarcoma cell type (1 case); (3) mixed cell type (4 cases); (4) lymphocytic type (2 cases). Stem cell and clasmatocytic lymphomas,[47] Hodgkin's disease, and follicular lymphoblastoma were not encountered.

In all forms there occurred in the imprint material occasional polymorphonuclear leukocytes, eosinophils, tissue mast cells and pathologic lymphocytes, histiocytes often with phagocytosed material, cells in mitoses, and lymphocytic cells other than those of the principal cell type. It must be emphasized that the cellular type peculiar to each case was universally found in all tissues and organs showing lymphomatous infiltrations. In addition, in those animals in which biopsies of lymph nodes were done, there was no evidence of cellular dedifferentiation as noted by Gall and Mallory[47] or of cellular differentiation as observed by Ehrlich and Gerber.[48]

1. *Lymphoblastic Type.* In imprints the cells of lymphoblastic type were round or oval, occasionally irregular, and varied from 9.12 to 18.42 μ with an average diameter of 13.68 μ (Fig. 1). The round or oval nucleus had a distinct membrane and ranged from 8.4 to 14.53 μ with an average of 11.18 μ. It was usually eccentrically located in the cytoplasm. The nuclear structure was leptochromatic with a very fine, regular chromatin network that presented a sieve-like appearance. The parachromatin was abundant and consisted of numerous minute, spherical granules imbedded in the chromatin. There were from 1 to 4 nucleoli, which in 2 cases were single and large with only an occasional cell containing multiple nucleoli. In the remaining 4 cases the majority of cells had smaller multiple nucleoli. In the former type the nucleoli were either central or peripheral, while in the latter they were irregularly scattered in the nucleoplasm. They were round or oval and the chromatin was condensed at their margins. The cytoplasm was moderately basophilic and Auer bodies and azure granules were absent. The blue-staining spongioplasm consisted of small, granular masses in the lighter hyaloplasm so that the cytoplasm appeared somewhat foamy, mottled, and vacuolated. The basophilic spongioplasm was usually more abun-

dant in the periphery of the cell so that it appeared darker, condensed, and homogeneous. A few small cytoplasmic vacuoles occurred in some cells and the perinuclear zone was often a lighter blue.

In sections the lymphoblasts varied from 4.93 to 11.1 μ and averaged 8.02 μ in diameter (Figs. 5 and 6). They were round or oval when loosely arranged and were often polyhedral and irregular when densely massed. The nuclei were round or oval, occasionally irregular, measured from 4.46 to 8.5 μ with an average of 6.39 μ, and were usually eccentrically located. The nucleus was vesicular in appearance with relatively few fine chromatin granules that were frequently marginated on the deeply staining nuclear membrane. All cells contained from 1 to 4 round or oval, prominent acidophilic nucleoli that often had accumulations of chromatin granules at their peripheries. The relative size and the distribution of the nucleoli were similar to those in the imprint material. The cytoplasm was homogeneous, vacuoles were occasionally present, and granules were absent. With hematoxylin and eosin the cytoplasm was amphophilic and with the Dominici stain it was blue.

2. *Lymphosarcoma Cell Type.* Imprints of cells of lymphosarcoma type stained with brilliant cresyl blue and Wright's stain revealed cellular characteristics that easily differentiated them from other cell types (Fig. 2). These cytologic features were less discernible with the May-Grünwald-Giemsa's stain or with Wright's stain alone. The round or oval cells measured from 8.78 to 14.4 μ with an average of 11.18 μ. The cytoplasm was moderately basophilic and occasionally contained small vacuoles. Azure granules and Auer bodies were absent and the perinuclear cytoplasm was often a lighter blue. The nuclei ranged from 6.73 to 12.0 μ with an average of 9.7 μ and were round, oval, or slightly indented. Two distinct types of nuclear structure existed with definite transitional stages between them. The nucleus of the immature lymphosarcoma cell was larger and had a leptochromatic structure similar to that of the lymphoblast. Other nuclei were more mature with heavy blocks of deeply staining chromatin of a pachychromatic appearance. Numerous transitional stages were observed between these two cell types and the majority of cells were of these intermediate forms. Each nucleus contained from 1 to 6 nucleoli that showed characteristic features with the brilliant cresyl blue and Wright combination. They stained a deep blue and were intimately surrounded by a narrow zone of deep purplish blue perinucleolar chromatin material. Application of the same staining procedure to cells of the other types indicated that the nucleoli did not stain in the same distinctive fashion and that nucleoli were absent in the lymphocytic type.

In sections the cells measured from 4.89 to 8.7 μ with an average of 6.56 μ and the nuclei from 4.63 to 6.51 μ with an average of 5.57 μ. In all detailed morphologic aspects, however, the lymphosarcoma cells appeared identical with the lymphoblasts (Fig. 7).

3. *Mixed Cell Type.* The imprints from cases with cells of mixed type revealed three distinct cell types that occurred in approximately equal proportions, although in some fields one or another cell form sometimes predominated (Fig. 3). These consisted of large lymphoblasts identical with those in the lymphoblastic type, small mature lymphocytes, and cells intermediate between these. The last were larger than the mature lymphocytes but were usually smaller than the lymphocytes. Their nuclear structure was immature but evidenced early pachychromatism and small nucleoli were only occasionally present. Measurements of approximately equal numbers of the three cell types indicated a variation of 6.4 to 17.2 μ with an average of 11.3 μ. The nuclei ranged from 6.35 to 13.7 μ and averaged 9.6 μ.

In sections the three cell types, namely, large lymphoblasts, mature lymphocytes, and transitional forms, were likewise present (Fig. 8). They varied from 4.59 to 14.66 μ with an average of 7.44 μ and the nuclei ranged from 4.0 to 10.78 μ with an average of 6.13 μ.

4. *Lymphocytic Type.* In imprints the cells of lymphocytic type measured from 4.97 to 10.15 μ with an average of 8.41 μ and the nuclei from 4.68 to 10.0 μ with an average of 7.55 μ. They had the morphologic appearances of mature lymphocytes as seen in the peripheral blood (Fig. 4). The nucleus was round or oval, occasionally indented, and nucleoli were absent. The chromatin was aggregated into coarse, block-like masses that contrasted with the parachromatin, producing a pachychromatic appearance. The nucleus was surrounded by a narrow zone of slightly basophilic cytoplasm that sometimes contained a few, small, azure granules.

In sections the cells were from 4.33 to 6.0 μ in diameter, with an average of 4.96 μ, and the nuclei varied from 4.0 to 4.88 μ with an average of 4.39 μ. Morphologically, the cells resembled the small mature lymphocytes as they appear in sectioned material (Fig. 9). The nuclear structure consisted of coarse, deeply staining, angular chromatin blocks; nucleoli were absent, and there was a thin rim of acidophilic cytoplasm.

Clinical Data

Incidence. In a group of 10,000 dogs, 20 cases of malignant lymphoma were encountered, representing an incidence of 0.2 per cent.

Age. The age varied from 5 to 12 years and averaged 9.08 years.

The disease thus occurs in older animals, as the normal dog usually lives from 10 to 14 years, barring death from accidents.

Sex. Of the 20 animals, 12 were males and 8 were females. This sex incidence differs from that found by other investigators, since in any larger group of cases females are rarely affected, as is true in man.

Breed. The breed incidence was as follows: Scottish Terriers, 7 cases; Boston Terriers, Cocker Spaniels, Chow Chows, 2 cases in each; Wire-haired Fox Terrier, German Shepherd, 1 case each; and mongrels, 5 cases. Although no definite conclusions can be derived from a small series, it appears significant that more Scottish Terriers were affected than the other breeds. This animal has become very popular in this country during the past 7 years but the increased numbers alone cannot be responsible for the increased frequency as other breeds, such as the Cocker Spaniel, occur in equal if not larger numbers.

Duration. It is obviously difficult, if not impossible, to determine the exact duration of the disease because of uncertainty as to its inception. In 8 cases a complete history was obtained from the owners, who stated that the animals were ill from 3 days to 5 months and averaged 43 days. Following the diagnosis, the dogs were hospitalized until death supervened, or was imminent so that the animal was killed. They survived from 13 to 64 days with an average of 38 days. In 5 dogs of this group the past history was known so that the total length of illness from its inception until death varied from 39 to 163 days and averaged 99.4 days. The course in our series corresponds with that reported by others although the disease when first recognized has in some instances lasted from 1 to 3 years.[7, 18]

Symptoms. In the early stages of the disease, the animals were apparently normal with the exception of "lumps" or swellings as reported by the owners. Later the dogs became less active and weaker, vomited occasionally, had diarrhea or were constipated, ate poorly; some showed thirst and polyuria, others coughed and breathed heavily. The temperature was normal although occasional slight elevations occurred. Hemorrhages, as reported by others as being similar to those in man, were absent. In the terminal stages, the animals were thin, cachectic, had purulent ocular and nasal discharge, and pale mucous membranes. Bilateral exophthalmus occurred in 2 cases, jaundice in 1, ascites in 2, and proptosed nictitating membranes in 4.

Observations upon the Blood and Bone Marrow

Table I summarizes the results of the blood and marrow examinations and the average normals [42] are included as a basis for comparison. Additional blood studies were made on 3 animals that were not autopsied and these showed changes similar to those listed in the table.

Peripheral Blood. In the early stages the red cells and hemoglobin of the peripheral blood were within the normal range. As the disease progressed, the erythrocytes and hemoglobin decreased so that a definite anemia existed in the terminal stages. Normoblasts sometimes occurred in considerable numbers and the red cells showed anisocytosis, polychromatophilia, poikilocytosis, and shadow forms.

In all animals except no. 4538 in which there was a leukopenia, the white cells were increased, sometimes to a considerable extent. This elevation resulted from greater numbers of nonsegmented and segmented neutrophils in most instances. Toxic changes were infrequent. With the exception of no. 7000, the relative number of mature lymphocytes was in the normal range or decreased. Lymphoblasts appeared terminally in large numbers in 1 animal only (no. 8847). Pathologic lymphocytes, identified by a pachychromatic nucleus without nucleoli, a deeply basophilic cytoplasm, and vacuoles in the cytoplasm and sometimes in the nucleus, occurred in 12 dogs. These cells, however, are not specific for the lymphomas as they are frequently present in the blood of normal dogs and in those with a wide variety of diseases. The other cellular types showed no noteworthy alterations and the blood platelets appeared normal. Changes of the blood suggestive of acute or chronic lymphatic leukemia as seen in man were absent in our cases.

Bone Marrow Examined by Biopsy. Differential counts of the aspirated marrow revealed lymphomatous involvement in 7 cases, in contrast to 11 cases in which lymphomatous involvement was determined by a study of sections of the bone marrow. This discrepancy will be discussed later in presenting the microscopic findings in the sections. In general, the aspirated marrow showed hyperplasia of myeloid cells, consisting principally of nonsegmented and segmented neutrophils, with an increase in neutrophilic myelocytes in several animals. In most instances the erythroid cells were decreased with consequent increase of the myeloid-erythroid ratio. The other cell types showed no noteworthy alterations.

Macroscopic Observations

No marked differences were observed in the gross pathologic changes of the different cellular types, so that all forms will be described together.

Lymph Nodes. With the exception of dog 4538, the superficial nodes were increased in size and varied from 1.5 to 9.5 cm. in diameter. This enlargement was usually bilateral and involved the cervical, mandibular, prescapular, axillary, peripenile, inguinal, and popliteal nodes. The degree of enlargement varied in the different cases and in the different nodes of the same animal although the cervical and mandibular groups were usually more prominent. They were nonadherent to the

TABLE I

Blood and Bone Marrow of Necropsied Dogs with Malignant Lymphoma

Type	Normal	Lympho-cytic		Mixed cell					Lymphosarcoma cell				
Case number		4538	7838	1474	2737	7000	7067		8318				
							1/26	2/24	9/17	9/25	10/1	10/9	10/13
Peripheral blood													
Red cells÷1000	6629	3370	4490	4020	3990	4080	7090	3670	3560	2760	3030	3530	3880
Hemoglobin (gm. per 100 cc.)	12.2	5.1	7.6	6.8	6.8	8.0	12.4	5.4	6.74	5.29	4.8	6.9	5.6
White cells÷100	135	25	768	836	515	199	586	330	214	190	216	204	460
Myelocytes		3.0					0.33						
Non-seg. neutrophils	5.3	65.0	12.2	18.0	25.4	7.0	27.99	29.5	14.0	14.5	12.5	24.0	36.0
Segmented neutrophils	67.0	14.0	66.0	39.5	56.4	39.0	66.66	50.0	68.0	74.5	61.0	66.5	43.5
Eosinophils	3.9		2.2		1.6			1.5		2.0			
Basophils	0.3											0.5	
Monocytes	2.8	1.5	11.2	6.0	2.6	2.5			1.5	2.0	4.0	2.0	2.0
Lymphoblasts											0.5	1.0	1.5
Lymphocytes	20.7	12.5	7.4	21.5	13.2	41.0	6.33	17.5	13.0	8.5	17.5	6.0	9.5
Pathologic lymphocytes	0	4.0	1.0	15.0	0.8	10.5	0.66	3.0	2.0	0.5	2.5	0.5	7.5
Normoblasts (no. per 100 white cells)	0.6	4.5	0.2	1.5	26	14.5			29.5	37.5	48.5	56.0	13.5
Bone marrow													
Cells per cc.÷1000	144				100				293	118	149	50	
Megakaryocytes per cc.	41.2				33				None	11	None	11	
Myeloblasts	0.58	3.4	0.6	0.2	0.2			2.0					0.4
Myelocyte neutrophils	3.76	17.2	10.2	7.2	2.2			22.6		1.6	0.4	0.4	2.4
Myelocyte eosinophils	0.26				0.2								
Non-seg. neutrophils	23.5	37.4	28.4	19.2	23.0			40.3	17.0	10.6	33.6	13.8	8.8
Non-seg. eosinophils	0.12	0.2									0.6		
Segmented neutrophils	18.5	0.6	24.0	3.8	38.6			8.3	4.2	10.8	7.8	37.2	5.6
Segmented eosinophils	1.56	0.4	0.4	0.6					0.8	0.4		0.2	
Segmented basophils	0.02												
Heterophils	0.02												
Megaloblasts	1.02	0.8	1.6	0.6				2.6		0.4			
Erythroblasts	2.5	1.2		1.2	0.8			0.3					
Normoblasts	35.18	20.6	10.8	11.4	24.6			11.6	62.0	13.8	21.4	8.8	2.8
Monocytes	1.2		0.4	0.2	1.4					1.6		0.4	0.8
Monoblasts	0.14												
Lymphocytes	9.8	12.6	22.2	54.0	8.4			12.0	4.0	18.8	17.0	27.4	20.0
Pathologic lymphocytes	0.04	1.8	0.2							0.8	0.2	1.6	2.0
Lymphoblasts		0.4	0.2	0.4					6.4	0.2	1.2		
Mature lymphosarcoma cell									2.2	29.6	9.6	7.8	46.8
Immature lymphosarcoma cell									2.8	8.8	7.4	1.8	9.2
Plasma cells	0.82	2.6	0.8	1.0	0.2					0.4			
Hematogones	0.44	0.4			0.4				0.4	3.0	0.8	0.6	1.2
Reticulo-endothelial cells	0.54	0.4	0.2	0.2					0.2				
Myeloid-erythroid ratio	1.36: 1.00	2.61: 1.00	5.90: 1.00	1:33: 1.00	2.52: 1.00			5.04: 1.00	0.35: 1.00	1.64: 1.00	1.98: 1.00	5.86: 1.00	6.14: 1.00

TABLE I (*Continued*)

Blood and Bone Marrow of Necropsied Dogs with Malignant Lymphoma

Lymphoblastic																	
6021	5829	8656		7760		6088				8847							
		1/26	2/15	4/24	5/15	3/11	3/23	3/27	4/9	4/18	4/25	5/2	5/9	5/17	5/24	6/7	6/18
6230	4850	6700	4170	4440	4370	4150	4440	4480	2505	6880	5960	6520	5100	4060	4650	4680	3800
11.3	7.4	11.8	6.9	7.0	6.8	8.78	9.7	7.6	3.1	12.3	11.0	12.0	9.2	7.8	8.6	8.2	6.1
167	155	154	216	255	195	557	679	707	214	210	193	104	112	169	167	188	372
24.5	10.5	12.5	14.2	14.0	44.0	4.5	8.5	14.0	10.0	17.5	13.0	6.8	5.8	13.0	15.5	15.5	9.0
65.0	78.0	76.5	80.8	78.5	53.0	71.5	62.5	55.0	82.5	71.0	72.2	65.8	62.6	50.4	54.0	61.5	52.5
	2.0	1.0							0.5		1.2	2.2	2.4	0.8		0.5	
—	4.5	2.5	1.8	2.5	1.0	4.0	5.0	1.0	4.5	5.5	5.0	6.2	8.8	7.0	6.0	2.5	8.0
						0.5						1.8	6.0	8.0	6.5	6.5	23.0
7.5	5.0	6.5	2.8	5.0	1.0	15.0	21.5	29.0	2.5	5.0	7.0	16.0	13.2	20.2	15.5	13.5	7.0
3.0		1.0	0.4		1.0	4.5	5.0	1.0		1.0	1.6	1.2	1.6	0.6	2.5		0.5
	1.0		0.4		1.0	1.0	0.5					0.2					
			101							24	23	12	55				
			33							None	None	None	33				
		2.2	3.4	0.4					0.5	0.2	1.0					0.2	1.2
1.2		6.2	3.0	2.6					10.0	1.2	1.6	1.0	0.6	1.8		0.6	6.4
		0.2								0.2				0.2			0.8
7.4		27.0	13.4	20.6					20.0	28.0	27.6	19.6	18.4	46.4		25.8	39.0
		0.4	0.2								2.0			2.0		0.4	0.6
0.8		24.4	66.2	21.6					5.25	45.8	40.4	53.6	15.8	13.0		11.6	8.6
		0.4	0.2	1.8						1.4	0.8	2.2	1.2	0.6		0.2	0.4
		2.2	0.6	0.6						0.2		0.2	0.6			0.2	0.2
		2.0	0.2	0.2						0.4			1.0	0.2		0.4	0.4
2.4		19.6	3.8	1.6					2.25	11.0	10.2	1.6	44.0	16.4		38.0	11.0
		0.8	0.8	0.2						2.0	5.0	3.8	3.0	2.4		1.2	0.8
53.0		13.0	7.2	47.8					60.0	8.8	8.4	14.6	10.2	10.6		14.2	18.6
											1.0	1.0	0.6	0.6		0.4	
35.0		0.2		1.2					1.75		0.8	2.2	2.6	2.0		4.2	10.6
0.2		0.4	0.6	0.8							0.8		0.2	0.6			0.8
		0.6	0.2								0.4	0.2	0.6	3.0		2.6	0.4
		0.4	0.2	0.6					0.25	0.8			1.2	0.2			0.2
3.91: 1.00		2.55: 1.00	18.78: 1.00	19.58: 1.00					15.88: 1.00	6.62: 1.00	7.19: 1.00	12.44: 1.00	0.78: 1.00	3.85: 1.00		1.00: 1.00	4.91: 1.00

skin, freely movable in the subcutaneous tissue, and were occasionally fixed to the underlying structures. The thoracic lymph nodes measured from 1.5 to 5.5 cm. and often encircled and compressed the trachea, bronchi, and esophagus. All the abdominal nodes were increased in dimensions, measuring from 1.5 to 9.0 cm. in diameter, with the greatest enlargements occurring in the sublumbar and mesenteric groups. The lymph nodes were usually discrete although adjacent ones sometimes appeared fused and matted together. They were a grayish tan and were moderately firm with a tense capsule. On sectioning a pale, watery fluid was often expressed. Some nodes cut with difficulty due to the softness of the nodal tissue. In several cases they were very soft and in 2 animals a few nodes were purulent.

Spleen. With the exception of those of 2 dogs, the spleens were greatly enlarged and measured from 23.5 to 41.0 cm. in length, 5.0 to 10.5 cm. in width, and 2.0 to 4.0 cm. in thickness. The edges were rounded and the capsules appeared tense and thinned. In 8 cases the spleens, on section, had the color, consistency, and appearance of raspberry jam, with innumerable spherical, pale gray nodules measuring from 0.2 to 0.3 cm. scattered throughout the pulp. In 3 spleens the pulp was a pale red, soft, and with similar nodules. In 2 animals (1 lymphocytic and 1 lymphoblastic) the spleens appeared normal. Several infarcts were present in 3 spleens. The enlarged spleens revealed an absence of the normal trabecular and pulp structure. There were several grayish tan nodules varying from 2.5 to 5.0 cm. in diameter in the subcapsular region in 2 spleens of the mixed cell type.

Liver. In 10 animals the liver was enlarged and in 3 instances it extended below the umbilicus. The edges were rounded and the surface was usually a light mottled red. The portal markings were rendered more prominent by innumerable minute, grayish nodules of pin-point to pin-head size, or by irregular, fine, grayish streakings.

Lungs. In 6 cases the lungs were congested and edematous. In 2 animals there were focal lesions of bronchopneumonia and in 5 they appeared normal.

Other Organs. In 1 case the posterior mammary glands showed areas of necrosis and purulent exudate. In 2 animals there were ascites, hydrothorax, and hydropericardium. In 1 case the small intestine just posterior to the duodenum was involved in a lymphomatous mass 7.6 cm. long and 5.1 cm. wide, with the intestinal lumen 0.5 cm. in diameter. The walls of the gallbladder were thickened in most instances. In all dogs the tonsils were enlarged, reddened, and protruded from their fossae. The membranae nictitantes were thickened and proptosed in 4 cases. Bone marrow examination was restricted to the ilium,

sternum, and ribs. Such marrow was a reddish color. The other tissues and organs showed no changes characteristic of the lymphomatous state.

Microscopic Observations

In general there were little, if any, differences among the various cellular types as far as the histologic involvement of the organs was concerned. All forms will therefore be considered together and any noteworthy variations will be mentioned.

Lymph Nodes. In most instances there was complete loss of architecture of the nodes so that the normal structure of follicles, trabeculae, and sinuses was over-run, obscured, and replaced by diffuse infiltrations of lymphoma cells. The capsule and perinodal fat were usually heavily infiltrated although in some nodes this involvement was light or moderate. These findings were also noted in nodes of normal size and in those slightly enlarged. The sinuses were sometimes persistent, particularly those of the medulla, and usually contained lymphoma cells. They were occasionally congested or edematous and fewer cellular elements were present. Infrequently there were focal areas of congestion and hemorrhage together with golden-yellow pigment (hemosiderin). Considerable hemorrhage involved several nodes of one animal. In most cases, one or more nodes showed focal areas of necrosis with polymorphonuclear leukocytic collections that were extensive in the definitely purulent nodes. Considerable variations were noted in the different cases and in the lymph nodes of the same animal in respect to the number of mitoses and of macrophages. Extramedullary hematopoiesis did not occur and an occasional megakaryocyte was seen among the infiltrations. Reticulum stains disclosed a relatively profuse, irregular argyrophilic network that encompassed groups of cells with no intercellular distribution (Figs. 11 to 13). In regions the reticulum was sparse, particularly in cases of the lymphoblastic and lymphosarcomatous cell types. The veins, lymph vessels, and rarely the arteries often showed mural infiltrations of lymphoma cells that were occasionally intravascular.

Dog 7760 deserves special comment as the lymph nodes regressed in size after the initial enlargement. The normal structure persisted despite the replacement of the lymphatic tissue with lymphoblasts. In two small nodes large numbers of lymphoblasts surrounded distinct follicles with secondary nodules.

The lymph nodes secured for biopsy were identical with those obtained at necropsy.

Spleen. The different spleens showed changes that could be classified into several categories irrespective of the lymphoma cell type. These

varied from no microscopic alterations (1 of lymphocytic type) to an organ that histologically resembled a lymph node (1 of mixed cell type). Between these extremes the spleens showed varying degrees of lymphomatous involvement. In practically all cases the capsule was markedly thinned and the trabeculae were reduced relatively in number. The lymphoma cells appeared as smaller and larger irregular nodules or as diffuse masses that often obscured and obliterated the usual architectural landmarks (Fig. 15). The malpighian corpuscles were generally absent although in some instances they were replaced by lymphoma cells. There were occasional small areas of necrosis in the central portions of the lymphoma cell groups. Between the nodular masses the pulp was often congested, hemorrhagic, and contained variable numbers of polymorphonuclear leukocytes and lymphoma cells, occasional plasma cells, histiocytes, monocytes, and frequently many megakaryocytes. The amount of hemosiderin, which is considerable in normal animals of this age group, was decreased in all cases except 2. In 5 spleens there were widespread areas of extramedullary myelopoiesis that consisted largely of myeloblasts, myelocytes, and band forms, a finding confirmed by the presence of these cells in imprints of the same spleens. In addition a few scattered areas of erythrocytogenesis occurred in 1 spleen of this group. The macroscopic presence of infarcts in 3 cases was confirmed by the histologic examination. The trabeculae and walls of the trabecular blood vessels commonly showed lymphomatous infiltrations with many cells assuming an intravascular position (Fig. 16). The splenic imprints contained, in addition to the cell types observed in the sections, relatively large numbers of pathologic lymphocytes that were identical with those noted in the peripheral blood.

Liver. In all cases there were portal lymphomatous infiltrations consisting of small and large irregular masses of perivascular distribution that involved from 10 to 70 per cent of the entire liver tissue (Fig. 14). The cells frequently invaded the walls of the veins and were occasionally intravascular although the arteries and bile ducts were normal. In addition, small lymphoma cell groups sometimes occurred in the sinusoids and around the central veins. In 4 cases there were moderately extensive areas of myeloid metaplasia. Megakaryocytes were commonly present in the capillaries. In the dog with jaundice, bile pigment occurred in the bile capillaries. The liver cells were well preserved although they occasionally showed mild fatty changes and were sometimes atrophic in the regions of heavy infiltrations. The sinusoids were congested and contained large amounts of hemosiderin

in several cases. In all animals there were heavy subcapsular infiltrations of lymphoma cells. An interesting observation previously described [49] is the reduced number of normally present intranuclear crystals in the liver while those of the kidney are in the normal range.

Gallbladder. The lamina propria, muscularis, and perimuscular connective tissue layer were heavily infiltrated with lymphoma cells while the mucosa and serosa were normal (Fig. 18).

Kidney. The recognition of lymphomatous infiltrations in the kidney is complicated by the fact that the dog frequently suffers a spontaneous interstitial nephritis [50] in which lymphocytic cells appear in the interstitial tissue. This disease is particularly common in animals of the age group in which malignant lymphomas occur. In interstitial nephritis, however, plasma cells occur, mitoses are rare, and there is often some fibrous connective tissue proliferation. In 5 cases (lymphoblastic and mixed cell types) there were several lymphomatous nodules of varying size in the corticomedullary junction and subepithelial pelvic connective tissue, and smaller perivascular collections in the cortex. The cellular collections were usually well localized and not extensive. The glomeruli, tubules, and blood vessels showed no alterations attributable to the infiltrations. Leukostasis was absent and in 3 kidneys there were several small areas of myeloid metaplasia.

Prostate. In older dogs the prostate often shows subacute and chronic inflammatory processes associated with lymphocytic infiltrations. As in the kidneys, recognition of lymphoma cells is difficult due to the pre-existent inflammatory cells. In the 6 male dogs on which autopsy was performed, lymphomatous infiltrations, usually as irregular nodules and less often as diffuse groups, occurred in the interstitial tissue in 2 cases (1 lymphoblastic and 1 mixed cell type).

Genital Organs. The testis, epididymis, and penis, and the vulva, vagina, uterus, and ovaries were free from lymphoma cells with the exception of the ovaries of 1 animal. In this dog there were diffuse nodular masses of lymphoma cells in the medullary portions of both ovaries.

Tonsils. In all cases the tonsils showed widespread infiltrations so that the normal structure was obscured (Fig. 19). The epithelial layer was flattened and lymph follicles were absent. Plasma cells, small lymphocytes, and neutrophils occurred in lesser numbers but macrophages were plentiful. The cellular infiltrations did not involve the mucous glands in the peritonsillar region.

Membrana Nictitans. The nictitating membrane of the dog normally contains distinct lymph follicles. In animals in which the third

eyelid was protruded, the follicular structure was replaced by diffuse infiltrations of lymphoma cells. Similar infiltrations were sometimes present in those eyelids that were not grossly enlarged.

Gastrointestinal Tract. Only 1 case (lymphocytic type) showed a gastrointestinal lymphomatous mass and that has already been described grossly. Microscopically, the intestinal coats were heavily invaded so that the normal structure was obliterated. The intestinal glands were fewer and the epithelium was ulcerated in areas. Despite the absence of macroscopic changes, Peyer's patches were usually replaced by lymphoma cells although there was no invasion of the neighboring structures. Changes characteristic of pseudoleukemia gastrointestinalis were not observed.

Pancreas. Lymphomatous infiltrations of the pancreas occurred in 5 cases of the lymphoblastic and mixed cell types. The cellular collections consisted of small, irregular nodules, principally confined to the interlobar connective tissue, that only occasionally infiltrated the intra-acinar connective tissue. The lymphoma cells were usually perivascular, often encircled ducts and ganglia, and sometimes occurred in the peripancreatic fat.

Lungs. In all cases the lungs showed more or less widespread, small and large nodular formations of lymphoma cells that were perivascular and peribronchial in distribution (Fig. 17). The vascular and bronchial walls were often invaded and lymphoma cells frequently occurred in their lumina. The interalveolar septa and alveolar lumina contained isolated lymphoma cells. Megakaryocytes were regularly present. The parenchyma was usually normal although congestion and edema and, in 2 cases, focal areas of bronchopneumonia occurred.

Adrenal. The adrenals showed lymphomatous infiltrations of various degrees in 9 cases. There were frequent perivascular nodules in the capsule and periadrenal fat that sometimes enveloped nerves and ganglion cells. Small cellular collections commonly occurred in the cortex, particularly in the zona reticularis and less often in the other zones. Widespread areas of myeloid metaplasia, limited to the fascicular and reticular zones, were present in 2 cases.

Eyes. In the dogs with exophthalmus there were large masses of lymphoma cells in the orbit that surrounded the optic nerve, muscles, and blood vessels but did not invade the periorbital tissues.

Bone Marrow. As previously stated, discrepancies were noted between bone marrow sections and the aspirated marrow concerning lymphomatous involvement. In the 2 animals in which lymphoma cells were absent, the marrow was hyperplastic in one and hypoplastic in the other. In the remaining 11 dogs the marrow showed scattered nodules

of lymphoma cells with a more diffuse infiltration in only 1 case. Surrounding the nodules were many megakaryocytes, few erythroid cells, and an extensive hyperplasia of myeloid cells (Fig. 10). The explanation for the discrepancies in the aspirated marrow probably lies in the fact that the biopsy needle may miss the nodules. Conversely, the needle may be inserted into a nodule so that numerous lymphoma cells will be aspirated and an erroneous high count obtained. The evidence indicates that the sectioned material gives a more accurate picture of the lymphomatous infiltrations in the bone marrow. Partial absorption of bone spicules, erosion of bone with extension to the periosteum, invasion of parosteal structures, and osteosclerosis were absent in our cases.

Other Organs. Microscopic examination of all other organs, including the brain and spinal cord of 3 animals, revealed no lymphomatous infiltrations.

DISCUSSION

In considering a suitable designation for the animal disease, several possibilities were weighed. Lymphoblastoma was rejected because there is no substantial evidence that these tumors arise from embryonal cells.[51] Pseudoleukemia in the original sense of Cohnheim[52] would be suitable although this term is often used without any specific meaning and includes Hodgkin's disease, lymphosarcoma, and many others. Lymphadenosis signifies a glandular structure and the generalized lymphosarcomatosis of Kundrat[53] shows more regional involvement with less frequent spread to the spleen, liver, and bone marrow. Sternberg's leukosarcoma[54] is objectionable as the blood changes are absent. Gall and Mallory's term,[47] malignant lymphoma, appears the most suitable despite its relative noncommitment regarding the detailed histologic alterations. This criticism is tempered, however, by considering the cytologic structure of the predominant cellular types.

Although numerous studies have been made of the lymphomas in dogs, there have been few, if any, attempts to classify these diseases from a cytologic viewpoint. The impression received from a study of the literature is that some lymphocytic cell is involved, often without any qualifying statements concerning its detailed structure. In man, on the other hand, many cytologic classifications of the lymphomas have been proposed, largely from studies of paraffin-imbedded, sectioned material. When comparisons are made of the same material prepared as imprints stained with the May-Grünwald-Giemsa stain, certain discrepancies are observed attributable to the definite loss of cellular details that the sectioned tissue undergoes in the fixing, dehydrating, and embedding processes. The advantages of dry imprint

preparations have been summarized by Kirschbaum and Strong [55] as follows: (1) All types of leukemic cells are present, rather than only those which gain entrance into the blood. (2) The cells are more easily classified than in sections. (3) The same criteria for morphologic identification of cells can be used here as in blood cells. The dry imprint method has been largely used by workers with experimental leukemia and only occasionally in investigations of human leukemia. Certain cells, such as the mature small lymphocytes, are equally recognizable in imprints and sections. When immature lymphocytic cell types are considered, the section method appears to be inaccurate unless imprints are studied simultaneously and used as a basis for comparison. For example, in our Figures 5 and 6 of sectioned lymph nodes of the lymphoblastic type, the cells resemble those which Gall and Mallory [47] term the stem cell type, and which Ehrlich and Gerber [48] denote as the reticular type. The imprint material of the same nodes indicates that these cells have the accepted morphologic characteristics of lymphoblasts and are probably not stem cells or reticular cells in the usual interpretation commonly applied to these terms. The lymphoblastic type of Gall and Mallory [47] does not resemble our lymphoblast and is probably a form intermediate between the lymphoblast and the mature lymphocyte. In our own material likewise discrepancies occur unless imprints are compared with the sections. The imprints of the lymphoblastic type shows cells (Fig. 1) which are entirely different from those of the lymphosarcoma cell type (Fig. 2). Sections, however, as depicted by Figure 5 of the former and Figure 7 of the latter type, are practically identical.

While conclusions derived from a study of animal material may have limited application to the human disease, the evidence strongly suggests that in the lymphomas, at least, there is definite loss of cytologic details in sectioned tissue. Such material should therefore be used as a means of cellular classification provided its limitations are known and preferably if lymph node imprints are studied simultaneously. It is essential to use absolutely fresh tissue, preferably surgical specimens, in preparing imprints, as autopsy material is unsuitable due to the associated autolysis.

In view of the hematologic findings, it is difficult to justify the use of the term "leukemia" in the canine disease. In our series and in the great majority of cases reported in the literature, the blood changes were not comparable to those occurring in human leukemia. The most characteristic blood alteration in addition to the anemia consisted of a nonspecific leukocytosis resulting from increased numbers of nonsegmented and segmented neutrophils. This blood picture frequently

has been considered as an expression of myeloid leukemia (Weil and Clerc,[9] Wirth,[18, 25] and others) and these authors have been extensively quoted to support the opinion that the dog develops this disease. Our blood studies, together with those reviewed in the literature, suggest that no bona fide instances of myelogenous leukemia have been described in this species. The mechanism for the leukocytosis is a speculative question and several possibilities can be considered. The presence of leukocytosis with malignant tumors in animals and man is well known and is usually attributed to the associated ulcerative and inflammatory processes.[51] Lewis [56] and others have demonstrated that the growing malignant tissue of dibenzanthracene tumors in mice produced an increased number of polymorphonuclear cells in the peripheral blood. Leukocytosis is often found in invasion of the bone marrow by malignant tumors. In the canine malignant lymphomas the leukocytosis probably results from a combination of these factors; namely, the marrow involvement, the necrosis in the lymphomatous tissue, and the relatively rapid growth of lymphoma cells. The hyperplasia of myeloid cells in the bone marrow adequately explains the neutrophilic leukocytosis encountered in the peripheral blood. Although the replacement of normal marrow by lymphoid foci is considered an important factor in the production of the accompanying anemia in man,[57] this explanation cannot be unreservedly applied to the canine disease. In the dog, the suppression of erythrogenesis results from the myeloid hyperplasia and only partially from the lymphomatous infiltrations which are both moderate and focal in extent. The anemia is probably similar to that occurring in many malignant tumors.

A consideration of the histologic alterations in the malignant lymphomas of dogs presents features that are of interest to the comparative pathologist. The aggressive tendencies, such as capsular and perinodal infiltrations and the nodular cellular masses in the different organs, point toward lymphosarcoma. On the other hand, the almost universal involvement of the spleen, liver, and bone marrow, in addition to diffuse cellular infiltrations, are not the typical findings that one usually associates with lymphosarcoma. While it is well known that the lymphomas are often difficult to separate microscopically and that numerous transitional forms occur, the malignant lymphomas in dogs, as here described, appear to be intermediate forms, irrespective of the cell morphology, between the pseudoleukemia of Cohnheim [52] and the lymphosarcomatosis of Kundrat.[53] The disease seems to partake of the histologic characteristics of both affections and in this respect resembles similar transitional types in man.

It might be of interest to consider the relative frequency of the

lymphomas in general as compared with other neoplasms occurring in dogs. Specifically, solitary lymphomas are relatively uncommon; in fact, in the experience of one of us (F. B.), only 1 such tumor was observed in a series of 196 neoplasms of all types. This growth was a primary reticulum cell lymphosarcoma localized in the right prescapular lymph node. The so-called lymphomata so frequently present in the spleens of older dogs cannot be placed in this category as they undoubtedly represent nodular hyperplasia and are not true neoplasms. The splenic "lymphomata" are analogous to the nodular hyperplasia occurring in the adrenals, liver, and pancreas of aged animals. The malignant lymphomas can thus be considered the most common tumor-like enlargements of the lymph nodes in dogs.

The fairly extensive areas of extramedullary myelopoiesis in 5 animals that involved the spleen, liver, kidneys, and adrenals are of interest. Such findings appear to be common in the canine malignant lymphomas as judged by the many reported cases of so-called myelosis with which the myeloid metaplasia has been confused. In man, on the other hand, extensive myeloid metaplasia is exceptional and has led to the question of a mixed or combined (myeloid and lymphatic) leukemia.[57] In dogs, however, the concept of a myeloid or mixed leukemia cannot be seriously entertained on the basis of the ectopic myelopoiesis in view of the universal presence of cells of lymphocytic type in the different organs. The heterotopic collections of myeloid cells probably arise from a combination of factors; namely, compensation for the partial replacement of myeloid tissue in the bone marrow by lymphoma cells and, as Lewis [56] has demonstrated in experimental tumors, the specific biologic features of some neoplasms that stimulate the formation of myeloid metaplasia. The extramedullary myelopoiesis occurred in the lymphocytic and mixed cell types only.

From the clinical point of view, the duration and course of the disease was subacute with little variation in the different cellular types. The usual subdivision into acute and chronic cannot be applied to our cases on the basis of cellular morphology or of duration of the disease.

Summary

1. The designation, malignant lymphoma, has been applied to a systemic disease of dogs in which the lymph nodes, spleen, and liver were usually enlarged. Microscopically, diffuse and nodular cellular infiltrations involved these organs as well as the bone marrow, adrenals, lungs, kidneys, prostate, tonsils, third eyelids, gallbladder, pancreas, and Peyer's patches. On a cytologic basis, the predominant cellular

types were classified into four distinct groups: lymphoblastic, lymphosarcoma cell, mixed cell, and lymphocytic.

2. Cellular identification as judged by dry imprints and sections of lymph nodes indicated greater accuracy for the former method. Therefore, the dry imprint method should be employed in classifying the lymphomas cytologically.

3. The peripheral blood showed anemia and usually a polymorphonuclear leukocytosis and is therefore not comparable to the true leukocythemia in human malignant lymphomas. Thus the term, leukemia, is inadmissible for the canine disease.

4. The widespread extramedullary myelopoiesis that is not uncommon has been frequently erroneously reported as myelogenous leukemia in the literature. Actually, no unquestionable cases of this disease have been described in dogs.

5. Clinically, the disease was subacute, irrespective of the predominant cellular type, in contradistinction to the usual findings in man.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

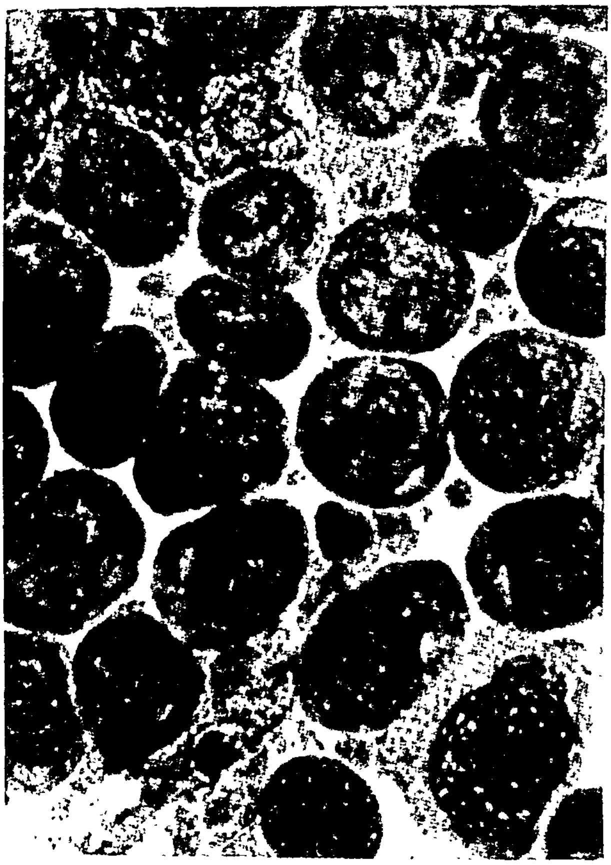
PLATE 117

FIG. 1. Lymph node imprint (case 8847, lymphoblastic type). The lymphoblasts have leptochromatic nuclei with large, prominent nucleoli. May-Grünwald-Giemsa stain. × 1320.

FIG. 2. Lymph node imprint (case 8313, lymphosarcoma cell type). The cells in the photomicrograph have a relatively mature nuclear structure and the prominent nucleoli are surrounded by a rim of deeply staining perinucleolar chromatin material. In other cells of this imprint the nuclei were more immature but the nucleoli were similar. Brilliant cresyl blue and Wright's stain. × 1320.

FIG. 3. Smear preparation of lymph node macerated in the serum of the same animal (case 1474, mixed cell type). The photomicrograph depicts four mature small lymphocytes, one lymphoblast (arrow), and four younger lymphocytes without nucleoli. Brilliant cresyl blue and Wright's stain. × 1320.

FIG. 4. Lymph node imprint (case 7838, lymphocytic type). The cells resemble the mature lymphocytes of the peripheral blood and contain pachychromatic nuclei without nucleoli. May-Grünwald-Giemsa stain. × 1320.



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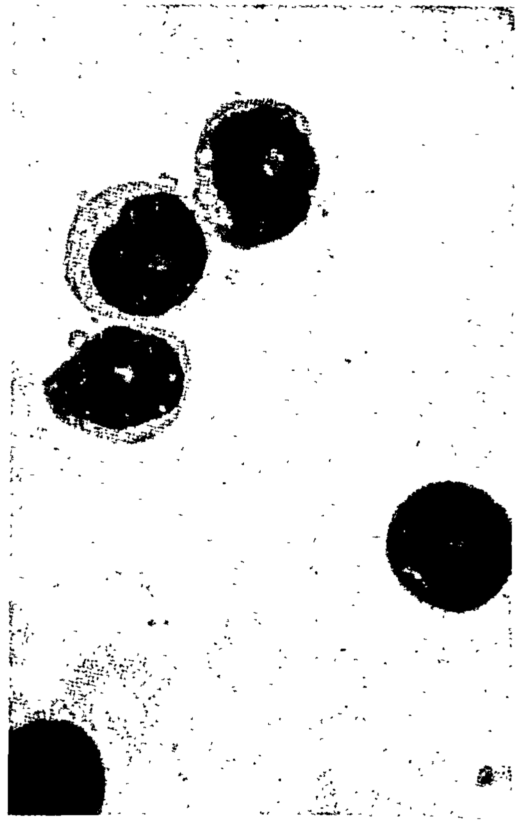

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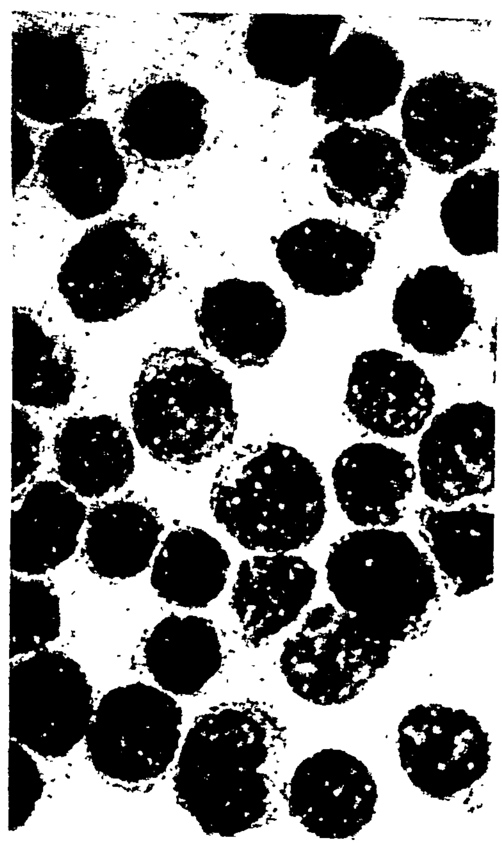

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Bloom and Meyer

Malignant Lymphoma in Dogs

PLATE 118

FIG. 5. Paraffin section of lymph node of the same case as shown in Figure 1. The lymphoblasts have vesicular nuclei and prominent single nucleoli. Dominici's stain. $\times$ 1320.

FIG. 6. Section of lymph node (case 6088, lymphoblastic type) in which there are multiple nucleoli and the same vesicular nuclear structure. The cytoplasmic boundaries are readily discernible as the cells are less densely packed in this field. Dominici's stain. $\times$ 1320.

FIG. 7. Section of lymph node of the same case as shown in Figure 2. From sections the lymphosarcoma cells in this figure and lymphoblasts of Figure 5 appear alike but differ in the imprints of the same cases as illustrated in Figures 1 and 2. Dominici's stain. $\times$ 1320.

FIG. 8. Lymph node section (case 7067, mixed cell type) showing lymphoblasts, mature small lymphocytes, and cells intermediate between these. Dominici's stain. $\times$ 1320.

FIG. 9. Lymph node section of the same case as shown in Figure 4. The cells are morphologically similar to mature lymphocytes as seen in sectioned material. Dominici's stain. $\times$1320.

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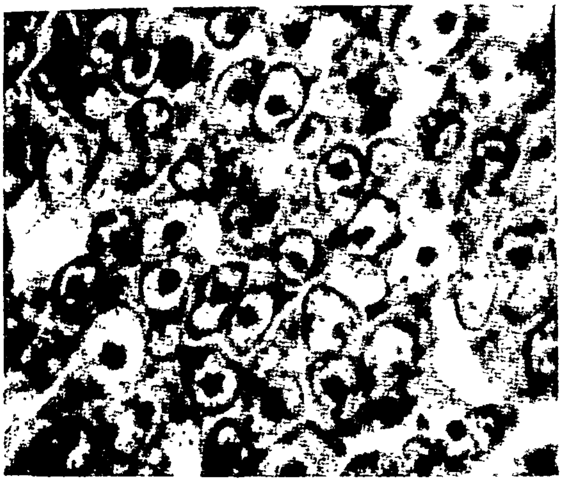

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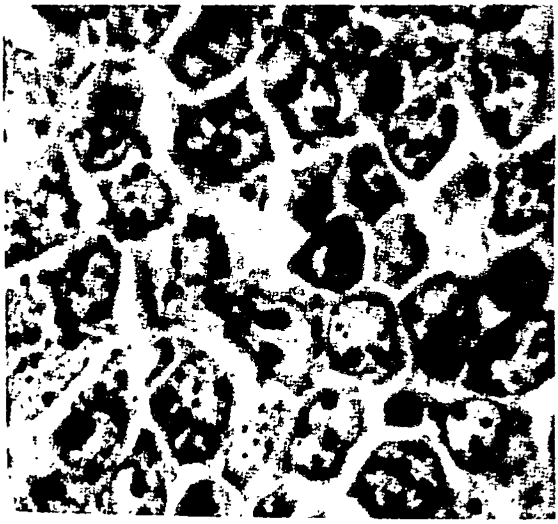

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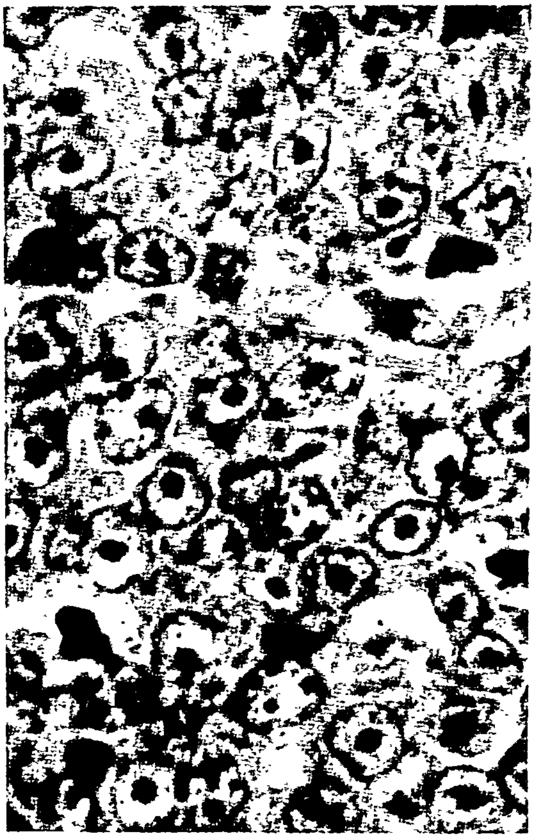

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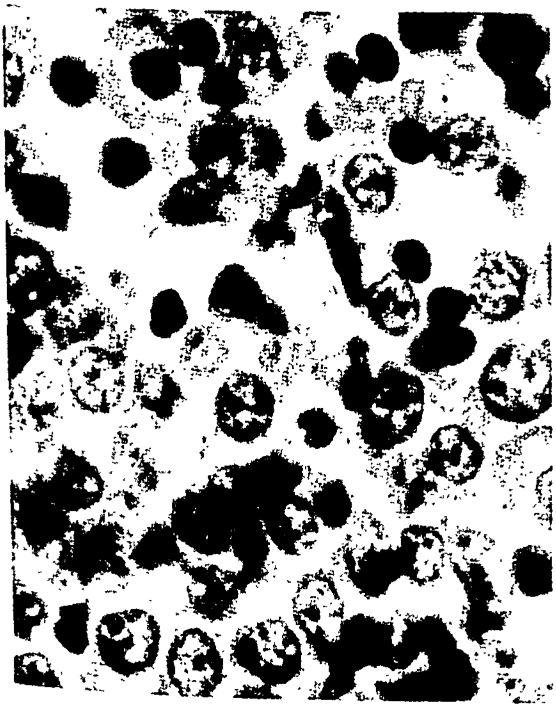

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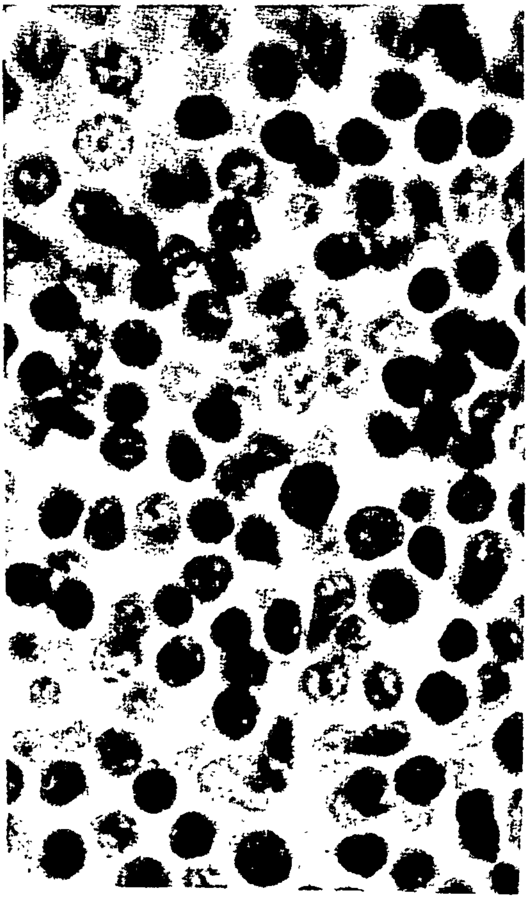

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PLATE 119

FIG. 10. Bone marrow section of case 6088 (lymphoblastic type). The right half of the photomicrograph is occupied by a lymphomatous nodule consisting of lymphoblasts. To the left, myeloid cells predominate and part of a megakaryocyte is present in the extreme upper left corner. Hematoxylin and eosin stain. × 880.

FIGS. 11, 12, and 13. Reticulum stains of lymph nodes of cases 6088 (lymphoblastic type), 7067 (mixed cell type), and 7838 (lymphocytic type) respectively. The argyrophilic fibers are similarly distributed in all 3 cases. Wilder's reticulum stain. ×293.

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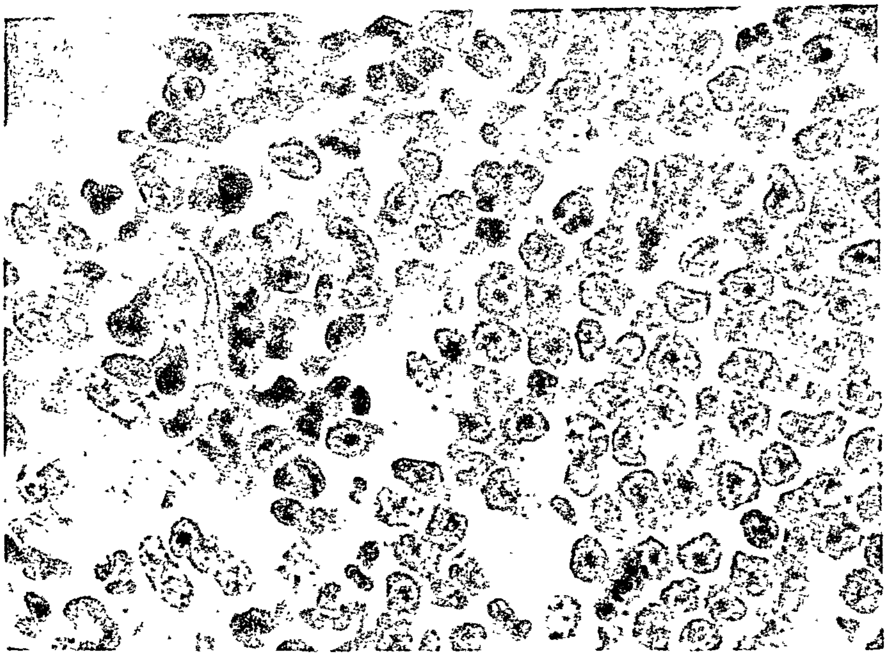

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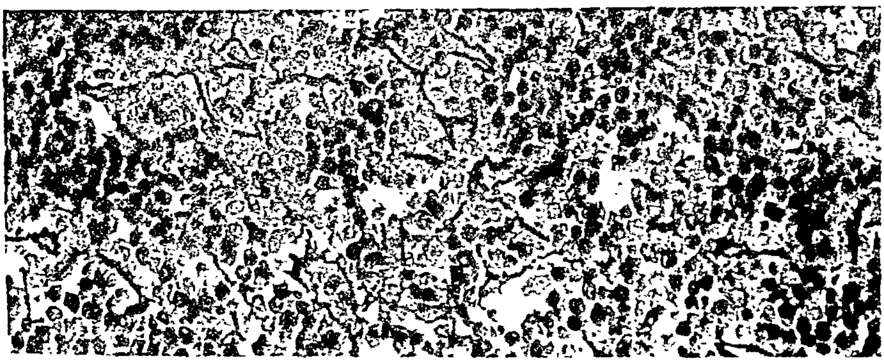

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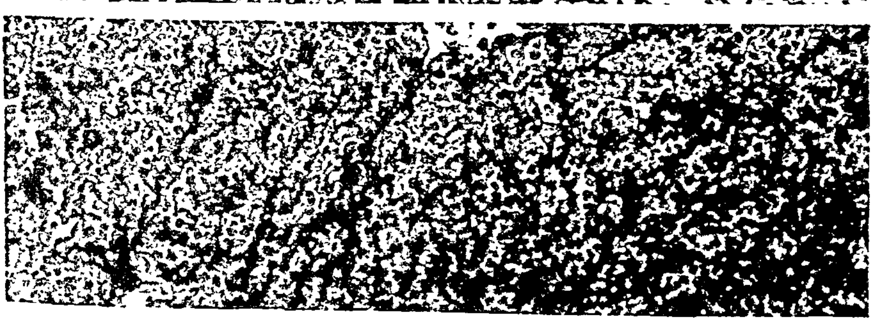

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PLATE 120

FIG. 14. Liver of case 7838 (lymphocytic type) depicting widespread portal and capillary lymphomatous infiltrations. In other cases the sinusoids were less extensively involved and the infiltrations were principally confined to the portal areas. Hematoxylin and eosin stain. × 88.

FIG. 15. Spleen of case 7760 (lymphoblastic type) illustrating a relatively diffuse infiltration of the pulp with lymphoma cells. Hematoxylin and eosin stain. × 88.

FIG. 16. Spleen of case 8847 (lymphoblastic type) with invasion of the trabeculae by cellular infiltrations. The lumen of the trabecular vessel contains a large lymphomatous nodule in addition to less compactly arranged cells. The dark staining granular material is hemosiderin. Dominici's stain. × 88.

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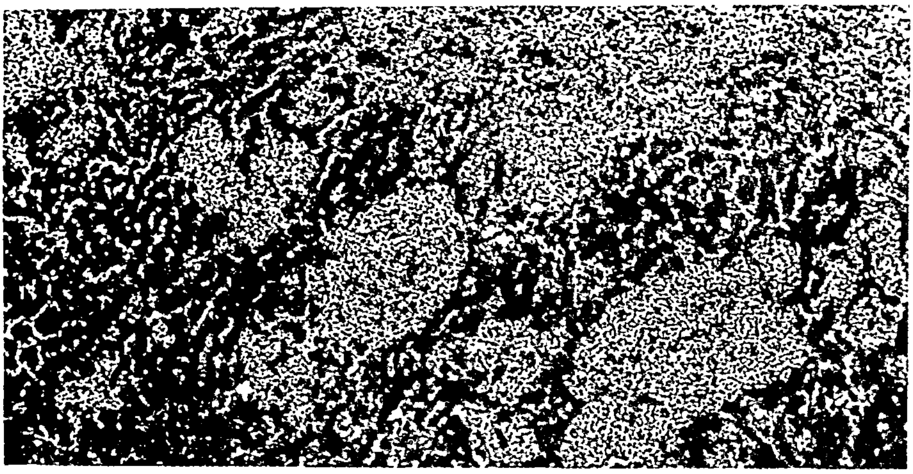

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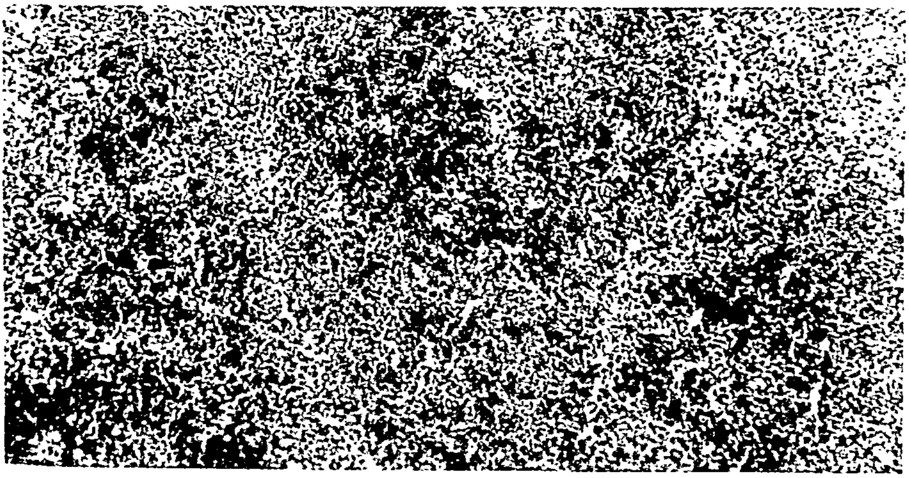

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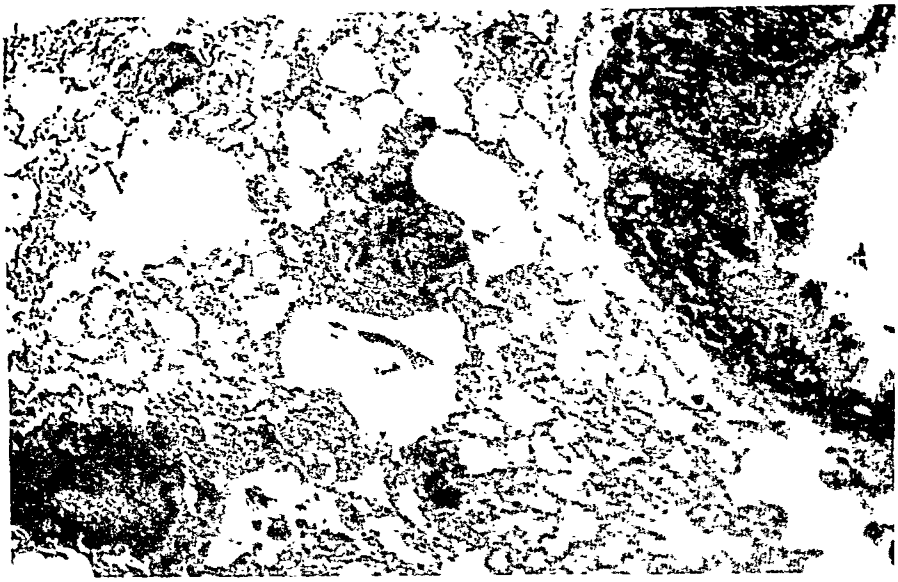
PLATE 121

FIG. 17. Lung of case 8847 (lymphoblastic type) showing lymphomatous nodules and invasion of the bronchial walls in the right half of the photomicrograph. Hematoxylin and eosin stain. ×88.

FIG. 18. Gallbladder of case 1474 (mixed cell type) depicting diffuse lymphomatous infiltrations. The epithelium is normal. Hematoxylin and eosin stain. × 88.

FIG. 19. Tonsil of case 6088 (lymphoblastic type) with diffuse lymphoma cell infiltrations, among which there are numerous macrophages. Hematoxylin and eosin stain. × 88.

17



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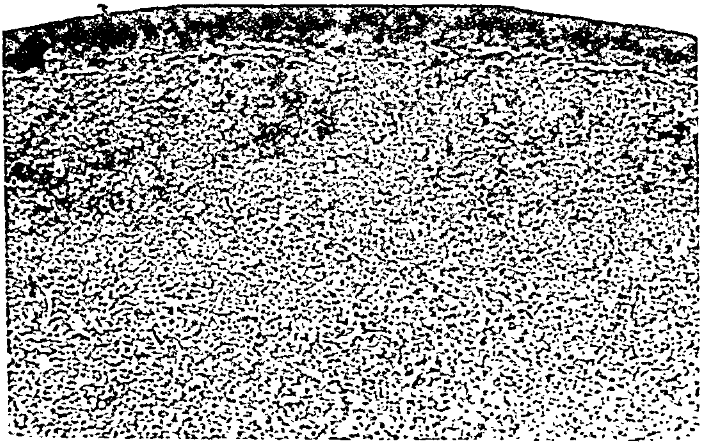

Bloom and Meyer Malignant Lymphoma in Dogs

THE INTERNAL LESIONS IN BURNS WITH SPECIAL REFERENCE TO THE LIVER AND TO SPLENIC NODULES

An Analysis of 96 Autopsies *

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The assertion by some [1, 2] that hepatic necrosis is usual in fatal burns has been countered by others [3] with the suggestion that the hepatic necrosis is due to tannic acid which had been applied in treatment. A desire to settle this issue caused me to examine a series of autopsies which had been performed on patients who had died following cutaneous burns. This led to observations on the state of the splenic nodules in burns and to an examination of the adrenals and some of the other viscera of special interest. The splenic nodules have attracted attention ever since Bardeen [4, 5] described well marked focal areas of degeneration in them which he interpreted as being indicative of a toxemia of burns. Weiskotten [6] described changes in the adrenal glands.

The problem of the internal lesions of fatal burns has been complicated in recent years by the use of local applications of tannic acid and other escharotics, by the giving of sulfonamides, and by the use of plasma and other intravenous fluids. Autopsies and reports made several decades ago should be examined with special care as they may actually be more reliable than autopsies performed recently. Fortunately, a portion of the autopsies which I examined came from this earlier period.

Material

Analysis was made of the cases of burns which had been autopsied at Duke Hospital and at Johns Hopkins Hospital.†

Only those cases were selected in which burns or the complications of burns appeared to be the principal factor leading to death. In reviewing the cases at Duke Hospital, complete protocols were prepared and all microscopic sections and the clinical histories were thoroughly studied. In reviewing the cases at Johns Hopkins Hospital I examined slides of liver, spleen, adrenal, and kidney in all cases in which these sections were available, and made notes of each case, as indicated in the sample protocol which follows:

* The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Duke University.

Received for publication, July 25, 1944.

† Permission to examine the autopsy protocols and slides at the Department of Pathology at Johns Hopkins Hospital was secured from Dr. A. R. Rich, and to examine the clinical records at Johns Hopkins Hospital from Dr. Winford H. Smith.

Colored male, 3 years of age (autopsy no. 15592). Death 2 days after burn. Two-thirds to three-fourths of body surface involved. Gentian violet spray. Temperature and pulse rose very high. No infection demonstrated in sections of skin. Spleen showed early acute splenic tumor. Liver showed slight diffuse fatty change.

In interpreting the histologic changes, the duration of survival is of great importance (Table I), for when death occurs after only a few hours it is conceivable that cells have already been damaged physiologically without cytologically demonstrable evidence of such damage. On the other hand, when death occurs several weeks after burning, cells which were necrotic at first may have been replaced by regeneration. Also, burns may become infected and bacteremia may develop in patients who survive longer than several days, and the changes observed in the internal organs may be the result of the bacteremia and not of the burn. Necroses in these older cases may be due to other complicating factors such as anemia and congestive heart failure.

TABLE I

Duration of Survival in Fatal Burns

Death during:	No. of cases
1st day	37
2nd day	9
3rd day	9
4th day	4
5th day	0
6th day	4
7th day	0
2nd week	7
3rd and 4th weeks	12
Period beyond 4 weeks	14
Total	96

Analysis of Table I showing duration of survival indicates that slightly more than one-third of the patients died during the first day, slightly less than one-third died during the remainder of the first week, and almost exactly one-third died after the first week. There were 26 cases in the most interesting period of all, that beginning with the second day and extending through the first week. It is during this period that deaths from burns occur with the least satisfactory explanations. Deaths during the first day are explainable on the basis of shock, especially when the burns are very severe, and deaths after the first week are usually explainable on the basis of complicating factors, but during the period from the second day through the first week primary shock has largely disappeared and infection usually has not developed.

Liver

Hepatic necrosis was not mentioned prominently in reports of fatal burns published before 1925, *i.e.*, before tannic acid was used on burns. In Schjerning's [7] table published in 1884 showing the occurrence of pathologic lesions following burns, the liver is not even listed. Dohrn [8] found no changes in the hepatic cells. McCrae [9] saw no necrosis in 12 cases. Marchand [10] stated: "The liver shows no essential finding. In high grades of blood destruction increased secretion of bile can occur, as in other types of hemoglobinemia." Weiskotten,[6] reporting 10 cases, found foci of necrosis of liver cells in 2.

Since 1925, attention has been called to severe hepatic necrosis in fatal burns, but apparently the severe grades of necrosis have been due to the tannic acid which was applied to burned areas. Vogt,[11] in 1929, reported 8 cases of fatal burns and found no necrosis of hepatic cells. He mentioned fatty change in these cells, and an increase and degeneration of leukocytes in the hepatic capillaries. In 3 of his cases death had occurred before 1925. There is no information as to whether tannic acid had been used in the treatment of the cases in which death had occurred after 1925.

In 1938 Wilson, Macgregor, and Stewart [1] described hepatic necrosis of severe grade in fatal burns following survival periods of from 2 to 12 days. (A photomicrograph of liver from one of their patients who died 71 hours after a burn shows extreme necrosis with only a rim of viable cells about the periportal areas.) Tannic acid had been applied to the burns in many of their cases. In 1939 Belt [2] described the hepatic necrosis following burns as similar to the hepatic lesions of yellow fever. In his 4 cases in which tanning with tannic acid had been employed, death occurred between the second and fourth days of survival. The necrosis is described as mid-zonal, but the illustration indicates that exceedingly few viable hepatic cells remain either about the central vein or about the periportal areas. Further evidence of the relationship between hepatic necrosis and the tannic acid therapy of burns [3, 12, 13] is summarized in an editorial in *The Journal of the American Medical Association*.[14] Wells, Humphrey, and Coll [3] were apparently the first to realize that the tannic acid was responsible for the hepatic necrosis rather than the burns, and they supported their view by producing hepatic necrosis in rats by subcutaneous injections of tannic acid.

Erb, Morgan, and Farmer,[15] in 1943, reported that of 27 patients tanned with tannic acid and dying in the period between the 3rd and 19th day, only 3 failed to show hepatic necrosis. The hepatic necrosis

illustrated by them is of extremely high grade, involving the hepatic lobule for nine-tenths of the distance from the central vein to the periphery. In strong contrast, no case of hepatic necrosis occurred in their 20 patients in the untanned group, 7 of whom died between the 3rd and the 14th days, a period in which necrosis was an outstanding feature in the tanned group; and no hepatic necrosis was seen by them in patients who had died before the introduction of tannic acid therapy for burns.

Observations

Necrosis was noted in the cases which I examined, but with relative infrequency, as indicated in Table II. Moreover, the necrosis was usually not at all severe. In only 3 instances was it as much as 2 plus,

TABLE II

Liver in Fatal Burns

Time of death in relation to injury	No. of cases with sections of liver	No. with necrosis	No. with fatty change
1st day	32	1 (±)	6 (+++, +, +, +, ++, +)
2nd day	9	1 (±)	2 (+, +)
3rd day	8	3 (±, +, +)	1 (+)
4th day	3	0	0
6th day	4	1 (±)	0
2nd week	6	1 (+)	0
3rd and 4th weeks	11	2 (+, +)	2 (++, +)
More than 4 weeks	14	4 (++, ++, ±, ++)	9 (+, ++, ++++, +++, +, +, ++, ±, +++)
	87	13	20

which indicated necrosis extending nearly one-half of the distance from the central vein to the periphery of the hepatic lobule. But all 3 patients had survived for more than 4 weeks, and necrosis could scarcely be thought of as the effect of the original burn, since cells originally rendered necrotic would have been removed unless, of course, a necrotizing influence from the burn continued to be present. Tannic acid had not been used in these cases.

In all 3 cases complications of the burns or other accessory factors explained the hepatic necrosis more reasonably than the direct effect of the burns. In the first case, a child of 1 year, there was purulent otitis media and thrombosis of cerebral veins. In the second case, a male, 66 years of age, there was thrombosis of periportal veins, pulmonary emboli, dilatation of the right side of heart, syphilis with gummata of lungs and testes and perforation of the palate, and bilateral lobular pneumonia. In the third case, a man, 52 years of age, a burn of the right arm extended to the bone and was treated at home for 11 days. Twenty-one days before death the patient was brought to the hospital

with thick exudate covering the arm. Phlebitis of the popliteal vein developed.

Five of the cases showed hepatic necrosis of a grade of 1 plus, indicating that necrosis did not extend more than a quarter of the distance from the central vein to the periphery of the liver lobule. Usually the extent was less. It occurred in 2 cases with death on the third day, in 1 case with death in the second week, and in 2 cases with death in the third and fourth weeks.

Analysis of necropsy and clinical data in these 5 cases indicated that in 1 case there was nothing but the burn to explain hepatic necrosis; in 1 case there were possible accessory factors, but the burn may have been the factor; and in 3 cases hepatic necrosis was explained best on complications such as infection or cardiac failure.

The following notes were made concerning the case in which there was nothing but the burn to explain hepatic necrosis:

Colored male, 41 years of age. Death on third day following burn in December, 1925.

Section showed central necrosis of the liver with polymorphonuclear neutrophils about the borders of necrotic cells. The central necrosis occurred in some areas of the section and not in others.

The anatomic diagnosis was: Burns. Central necrosis of the liver.

The clinical record indicated that the patient was burned over one-half of the body surface 3 days before death. There was no note indicating the presence of jaundice, and no evidence that tannic acid was ever applied, and there was a note that vaseline gauze had been used. The pulse rate was 100 to 130 per minute. Death occurred following urinary suppression.

Five cases showed probable hepatic necrosis, as indicated by a plus-minus sign. In the sections of liver from these cases there was mild condensation of the nuclear material in the cells closely surrounding the central veins, such as to suggest pyknosis, but no corresponding cytoplasmic change; or there was some other slight change of questionable significance.

In 2 of these 5 cases with equivocal necrosis there was no factor but the burn to account for the change noted microscopically.

When Table II is reconstructed with omission of the cases in which hepatic necrosis is better explained by complicating factors, it appears as in Table III.

Thus, hepatic necrosis of high grade did not occur as a direct result of burning. Necrosis of minimal grade was present in four instances in which the burn may have been the cause. It was noted with death on the first day in one instance (of plus-minus grade), and with death on the third day in three instances. Hence, minimal degrees of hepatic necrosis may apparently be caused by burns; and it seems probable that the changes observed were not present in the liver before the burns

occurred, as the nearly complete absence of necrosis in the large group of patients dying during the first day suggests.

How was the minimal necrosis produced? The untoward effects of therapeutic agents may have been responsible. Tannic acid was not applied in any of these 4 cases. (In another case, not included in the revised table, necrosis may have been due to tannic acid. This is discussed later.) Sulfadiazine spray was used in one. Plasma and other intravenous fluids might be responsible, since the older reports mention necrosis so infrequently. Other theories include the acute congestion of shock, absorption of toxic substances from the burned area, and thrombosis. No support for the last theory was obtained from microscopic study.

TABLE III

Liver Necrosis in Fatal Burns

(Necrosis Due to Complicating Factors Excluded)

Time of death in relation to injury	No. of cases with sections of liver	No. with necrosis "due" to burn
1st day	32	1 (±)
2nd day	9	
3rd day	8	3 (+, +, ±)
4th day	3	
6th day	4	
2nd week	6	
3rd and 4th weeks	11	
More than 4 weeks	14	

Hepatic Fat. Histologically demonstrable fat was noted most frequently in those who died on the first day and in those who died in the period beyond 4 weeks. When it was noted in those who died on the first day, the question arises as to whether the fat was present before the burn as a storage phenomenon or whether it indicates hepatic injury. This question cannot be answered without control studies. When the fat was noted in those who died in the period beyond 4 weeks, the question arises again as to whether the state of nutrition of the patient accounted for the fat or whether the fat indicated hepatic damage. In some instances in which the fat bordered the necrotic areas it seemed clear that it indicated hepatic damage, but in the numerous instances in which fat was present without necrosis the fat was not interpreted as an indication of hepatic damage.

Tannic Acid

Apparently the tannic acid therapy of burns never had any great vogue at Johns Hopkins Hospital or at Duke Hospital. Some of the Hopkins cases were autopsied before the advent of the tannic acid treatment, and it was possible to study these early cases with certain

knowledge that tannic acid was not utilized. Twenty-eight of the Hopkins cases occurred before 1924. Davidson's [16] paper, "Tannic Acid in the Treatment of Burns," appeared in August, 1925; and the cases which he studied occurred in and after May, 1924. Of the 28 cases, sections of liver were present in 21, all of which were normal except 2. The protocols of the 7 cases without sections indicated the probable absence of hepatic necrosis in these cases also. Of the 2 cases, 1 case, previously mentioned, showed 2 plus hepatic necrosis probably associated with secondary infection of the burn, death occurring 32 days after a small burn. In the other case there was questionable necrosis; and confluent lobular pneumonia with toxemia, or with cardiac failure, offered as satisfactory an explanation as did the burns for the slight changes noted in the liver. Hence, in a series of 28 cases from the period when tannic acid was not used there was no case of hepatic damage due to the direct effect of the burns.

In the cases at the Johns Hopkins Hospital after 1924 it was not determined in each instance whether tannic acid had been used, since analysis of the clinical records was impracticable. But in all those cases in which hepatic necrosis was observed microscopically the clinical record was thoroughly reviewed in attempting to make certain whether tannic acid had been used. In only 2 of the 13 cases of Table II which showed necrosis microscopically had tannic acid been used. In 1 of these, the child was treated with tannic acid compresses. He died on the second day and no condition other than the burns was discovered. The minimal necroses in this case may have been due to the absorption of tannic acid. In the other case a girl of 9 years was treated with tannic acid over a burn of more than half of the body surface sustained 44 days before death. The minimal necrosis of the liver could not have been due to the tannic acid, because of the long interval of time; and there were other factors, such as profound anemia, which could have produced the hepatic change. Hence there is one case in this series in which hepatic necrosis may have been produced by tannic acid.

The problem may be approached, also, by determining what the liver showed when it was known that tannic acid had been used. This was true in at least 2 of the cases at the Johns Hopkins Hospital. In 1, previously mentioned, death occurred on the second day. The equivocal necrosis may have been due to tannic acid. In the other, tannic acid had been applied to second degree burns of the back and buttocks of a colored boy of 18 months. Death occurred 12 hours or so after the burn. The liver appeared normal, but with such early death the general effects of tannic acid would probably not be histologically apparent.

Tannic acid had been applied to two of the burned patients autopsied at Duke Hospital. Death occurred 30 days and 44 days after burning and no necrosis or scarring was noted in either case. At such long intervals after the burn any original necrosis might have been replaced by regenerated liver cells.

Concerning tannic acid, then, the present series gives little information except in comparison with the series of others, such as those of Wilson, Macgregor, and Stewart,[1] Belt,[2] or Wells, Humphrey, and Coll,[3] in which tannic acid was used extensively and in which necrosis of high degree was noted when death occurred during the first few days after the burns were received. The comparison indicates that hepatic necrosis in the cases of these authors was the result of the tannic acid treatment rather than of the burns. Experimental studies of Wells, Humphrey, and Coll, of Baker and Handler,[17] and of others, show clearly that hepatic necrosis can be produced regularly in experimental animals by the application of tannic acid to a wound or by subcutaneous injection of tannic acid.

Splenic Nodules

Bardeen [5] described swelling and necrosis in the lymphoid nodules of the lymph nodes, peripheral lymphoid tissue, and spleen which he considered characteristic of burns and indicative of toxemia, just as similar changes in diphtheria were indicative of toxemia. Regarding the spleen he stated:

> "The Malpighian bodies become enlarged from the swelling of their cells and from the subsequent degenerative and necrotic process which begins at the centre. Single cells and small groups of cells may be seen degenerating in all parts of the pulp, but well-marked focal areas of degeneration are confined to the centres of the Malpighian bodies."

These observations were made on the organs of five children who had been fatally burned. The children varied in age from 16 months to 8 years. The time elapsing between burning and death varied from 4 to 9½ hours. He commented on the fact that in the child who died soonest, and only 4 hours after the burn, the lymphatic lesions were well marked, but he did not suggest that the changes might have been present before the burn was sustained. In a footnote Bardeen [4] stated:

> "Since working up the cases described above I have had the opportunity of studying the tissues from the bodies of two adults whose death was due to superficial burns. In each case lesions similar to those described above were found, but the lesions seemed less marked in the lymphatic tissue of the adults than they were in those of the children."

McCrae [9] did not find the constant presence of focal necrosis in the lymphatic glands, upon which Bardeen laid considerable stress.

Dohrn [8] did not note in his cases the full development of the changes described by Bardeen. He recognized that Bardeen described the changes in children and he questioned whether the changes were specific for burns.

Weiskotten [6] reported on 10 cases of burns, 5 in children and 5 in adults. Regarding the spleen he stated:

"Microscopically, in all of the cases of less than 3 days' duration there were found rather characteristic lesions in the lymph nodules. . . . There was apparent necrosis of the cells of the germinal centers evidenced by karyorrhexis. Endothelial leukocytes . . . were phagocytic for the necrotic cells and for the lymphoid cells of the nodules. . . . In many instances this process continued until the lymph nodule was represented by a large central area filled with phagocytic endothelial leukocytes surrounded by a narrow rim of lymphoid cells. These lesions apparently developed very soon after the burns were received, and were evident in all of the cases of less than three days' duration. In the cases of more than three days' duration, there were areas corresponding in distribution to the lesions described in the earlier cases. These areas were relatively homogeneous and eosin staining with occasional vesicular nuclei. At the periphery of some of these were seen occasional cells resembling the endothelial leukocytes seen in the earlier lesions. These appearances suggest that the areas represent the earlier lesions in process of hyaline degeneration and resolution or repair."

Lubarsch [18] considered the changes in the lymph nodules to be the most constant finding in uncomplicated fatal burns. He noted that the change occurred in the great majority of instances in children, and pointed out that in children the lymphatic tissue reacts especially vigorously in the most varied diseases. Vogt [11] stated that in children the foci were regularly present. Fender [19] was interested in the changes in the lymphoid nodules as indicating toxemia. He mentioned the similarity of the changes in infections and intoxications such as scarlet fever and diphtheria.

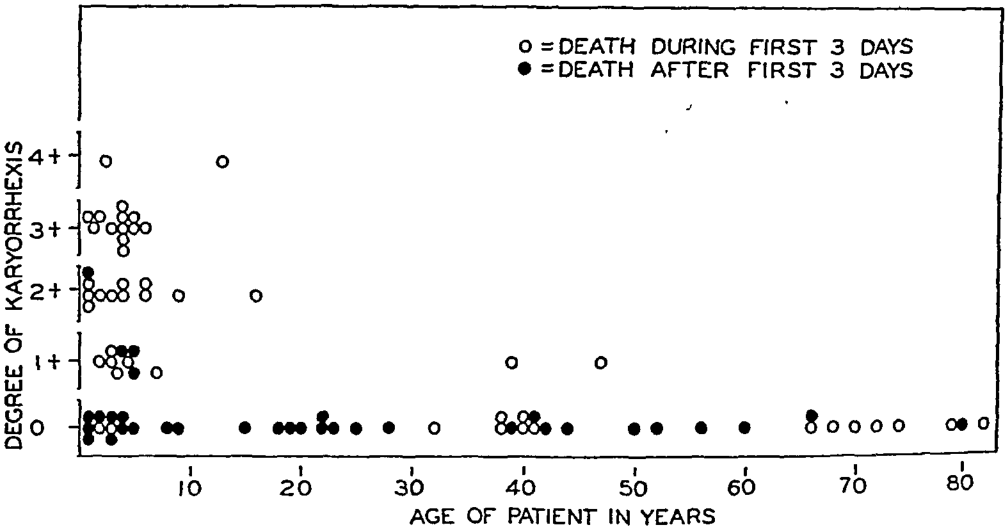
Observations

In the observations which I have made I have concentrated on a single, easily recognized feature of the changes described in the secondary centers (germinal centers) of splenic lymph nodules, or, more accurately, lymph cords. This feature is karyorrhexis (nuclear fragmentation). Usually when much karyorrhexis was present the secondary centers were large. The degree of karyorrhexis is expressed from 0 to 4 plus, varying from no observable karyorrhexis to the highest amount noted. If the degree of karyorrhexis so observed is correlated with the age of the patient (Text-Fig. 1) it is obvious that high degrees of karyorrhexis are noted only in the young and almost exclusively in persons less than 17 years of age. More than a 1 plus degree of karyorrhexis was not encountered in a single subject beyond adolescence.

In Text-Figure 1 the cases are divided into two groups depending on

whether the survival period was more or less than 3 days. It is noted that in only a single case with death after the third day was the degree of karyorrhexis as much as 2 plus. Thus the young (*i.e.*, those less than 17 years old) who died more than 3 days after receiving a burn failed to show the high degree of karyorrhexis which was exhibited by the young who died within 3 days.

The significance of these clear-cut results is difficult to evaluate, but several aspects may be discussed. It might be concluded that the burn is the cause of the high degree of karyorrhexis in the young. However, it may be that this degree of karyorrhexis was already present in the



Text-Fig. 1. Correlation of the age of the burned patient, duration of survival following burning, and the degree of karyorrhexis in the germinal centers of the splenic nodules. Each dot or circle represents a case. High degrees of karyorrhexis in the splenic nodules in fatal cases of burns occurred only in the young, *i.e.*, in those less than 17 years of age. In addition, high degrees of karyorrhexis occurred only in those who died during the first 3 days following the burn, regardless of age.

young, and that the burn which killed the child merely permitted the pathologist to see something normally present in the spleen. If this were so, it was also true that the condition present "normally," and during the first 2 days after the burn, disappeared some days later, possibly due to inanition, infection, or some other factor.

To try to determine the normal degree of karyorrhexis in the splenic nodules, sections of spleens were obtained from persons who had died within 24 hours after accidental injury such as an automobile accident, shooting, stabbing, strangulation, or suffocation. In many cases death was instantaneous. Only subjects at least 1 year of age were used.

In several thousand autopsies, 54 cases of such violent deaths were found, but unfortunately only 2 of these cases were young children

(5 and 3 years of age). These showed 1 plus karyorrhexis. Two plus karyorrhexis was shown by 2 adults, aged 21 and 45 years. None showed 3 plus or 4 plus karyorrhexis. Thirty-nine showed no karyorrhexis and among these there were no children less than 10 years of age. Hence one obtained the impression that normally the splenic nodules do not exhibit a high degree of karyorrhexis, but obviously the number of children in the series is too small for a conclusion. It would be desirable to examine a large series of accidental deaths in children, with death occurring soon after the accident, in an institution where such autopsies are available, in order to determine the degree of karyorrhexis present normally.

Karyorrhexis was observed within a few hours after a burn. Karyorrhexis of 3 plus degree was noted in a child who survived only 3 hours, and karyorrhexis of 4 plus degree was noted in a child who survived only 6 hours. Thus if the burn is the cause of the nuclear fragmentation the lesions develop with great speed. It may be that nucleated cells of the blood or of the tissues are damaged by the burn, just as red cells are,[20] and that particles of the injured nuclei are brought to the splenic nodules and deposited there. Or it may be that the cells of the lymph nodules are damaged by a toxic substance brought to them in the blood. Attempts to determine the origin of the nuclear particles by microscopic study were not successful. Some of the particles suggest the lobes of necrotic polymorphonuclear cells while others suggest pyknotic lymphocytes. Usually the particles are within the cytoplasm of macrophages. Occasionally the macrophages of the secondary centers of the splenic nodules also contain portions of red blood cells.

The failure to find karyorrhexis of high degree more than 3 days after receipt of the burn may indicate that sufficient time has elapsed to permit the macrophages to digest the nuclear material, or at least to destroy its property of absorbing the basic stain. This is in accord with the interpretation of Weiskotten.[6]

Kidneys

Sections of kidney from 88 of the cases were examined. A small number, only 9 cases, showed definite recent lesions. Infections, resulting from the burns, were present in the cases with renal lesions in which death occurred in the second week or later. These infections constituted a thoroughly satisfactory cause for the lesions noted. If these cases were excluded, only 4 remained in which renal damage was present and might have been a direct effect of the burn. In 1 of these 4 cases in which death took place 12 hours after the burn there were

hyaline droplets in the tubular epithelium, but diabetes was a complicating factor. In the 3 cases with death on the third day the renal changes might well have been due to the burn itself, but in all 3 cases there were complicating factors also, which might have been responsible for the changes observed. The type of damage and attendant circumstances in these 3 cases with death on the third day are given in the three succeeding paragraphs.

In the first case there was necrosis of the renal tubular epithelium of a moderate grade, with no obstruction of the collecting tubules. A third degree burn of four-fifths of the body surface had been sustained, hematuria had been present, and sulfadiazine spray had been used locally and plasma intravenously.

In the second case there was patchy necrosis of renal tubular epithelium. Epithelial cells were dislodged and had been caught in the tubules farther down. Hyaline droplets were present occasionally in the tubular epithelial cells. Extensive burns had been sustained and lobular pneumonia had developed.

In the third case there was minimal dilatation of the convoluted tubules. There was also focal necrosis of liver and hemorrhagic ileitis and colitis in this subject of 68 years.

No such spectacular lesion as that described by Brown and Crane,[21] bilateral cortical necrosis, was encountered in any of the cases in the entire series.

The cases at Johns Hopkins Hospital were examined before I knew about the importance of hemoglobinemia and hemoglobinuria in burns,[20] and I probably did not examine with sufficient care the contents of the tubules for the presence of hemoglobin casts. The lesion in the distal convoluted tubule and elsewhere in the kidney which has been described recently for the crush syndrome [22] and also in patients with burns [23] may have been overlooked.

The cases at Duke Hospital were re-examined for hemoglobin casts and changes in the distal convoluted tubules. Seven cases with patients surviving a short time were available, death occurring after 3, 12, 14, 22, and 40 hours, and 4 and 8 days, respectively. In 2 cases there were appearances suggesting hemoglobinemia, though this had not been established clinically.

The first of these was a woman, 39 years of age, who had received deep charred burns over 60 per cent of the body surface. Plasma (2300 cc.), whole blood (100 cc.), and cortin had been given. Death occurred 14 hours after the burn was received. At autopsy the bladder was empty. Blister fluid was pink as was much of the subcutaneous edema fluid. The intima of the aorta was stained intensely red. In the

blood vessels of the kidney distorted, shrunken, or conglomerated red cells occurred. The shrunken forms were identical to those described in the peripheral blood by Shen, Ham, and Fleming.[20] In the capsular spaces of the glomeruli eosin-staining material occurred which suggested hemoglobin. In an occasional collecting tubule there was eosin-staining fluid, débris, and rounded masses suggestive of hemoglobin.

In the second case, a child, death occurred at the end of 4 days. Burns were extensive and severe and had been treated with dry dressings. Oliguria and azotemia developed. In the distal convoluted tubules granular débris and masses with the eosin-staining qualities of hemoglobin were present, but there was no necrosis of the epithelium of the tubules. In a very rare collecting tubule débris which looked like hemoglobin was present.

In summary, even with these added considerations, renal changes in the series of burns were not impressive, and changes noted in patients dying in the second week or later were explained best on the basis of the secondary infection and septicemia which was usually present. In 3 cases, with death on the third day, it is possible that the changes were the direct result of the burns, but even in these cases there were complicating factors. Hemoglobin casts would undoubtedly have been found in some of the Hopkins cases upon re-examination.

Adrenal Glands

According to Weiskotten [6] the most prominent and characteristic of the necropsy findings in patients with burns are the changes in the adrenals. He described swelling, redness, and periadrenal edema with hemorrhage in all cases of more than 24 hours' duration. The weight of the adrenals in one of his cases was three times normal. Microscopically there were congestion and hemorrhage, and the gland cells were pale-staining and much swollen. Necrotic gland cells being invaded by polymorphonuclear and large mononuclear cells were not infrequent.

Observations

In the majority of cases the adrenal gland did not show arresting changes grossly, and the organ was usually described as normal, or congested, or as showing periadrenal edema.

I examined sections of the adrenal glands stained with hematoxylin and eosin from 68 cases. An analysis of the microscopic observations indicated the following: (1) Most of the cases showed no impressive change in the adrenal; (2) Congestion in and about the adrenal, periadrenal edema, and rarely periadrenal hemorrhage (not massive hemorrhage) were noted in several cases, especially during the first 3

days; (3) Necrosis was noted in 2 cases and was due to infection. It was thought that the congestion, periadrenal edema, and occasional periadrenal hemorrhage were probably features of shock and of the shifts of fluid which occur in burns. It is to be borne in mind, however, that changes in the adrenal of a functional metabolic nature could not be evaluated with any degree of refinement by simply looking at a section of adrenal gland stained with hematoxylin and eosin. Study of fat stains, granule stains, and quantitative methods might reveal changes.

Since the observations in the preceding paragraphs were made I have noted two pertinent references concerning adrenal lesions. Mallory and Brickley [24] reported focal necrosis of the adrenal in 2 cases in which death followed the Cocoanut Grove fire by 2 days in one instance and by 3 days in the other. They mentioned splitting of the cords of the outer portion of the cortex with accumulation of serous exudate in the space produced, pyknotic nuclei, acidophilic necrosis of adrenal cells, and infiltration of polymorphonuclear cells. Rich [25] has recently described a peculiar type of adrenal cortical damage associated with acute infections and has discussed the possible relation of this damage to circulatory collapse. The lesion consists of necrosis of isolated cells and a striking transformation of the solid cords of the zona fasciculata into tubular structures containing an inflammatory exudate.

In view of these observations, sections of adrenal were re-examined in cases autopsied at Duke Hospital with death occurring 3, 12, 14, and 40 hours, and 8 and 20 days following burns. In all of these cases, except in that with death on the 20th day, shrunken, dark-staining, apparently pyknotic nuclei were found occasionally in the cells of the cords of the zona fasciculata; and in 1 case, with death at 40 hours, some cells of the zona fasciculata had intensely eosin-staining cytoplasm in addition to pyknotic nuclei. Accumulations of inflammatory cells were not seen about the cells with intensely staining nuclei. I am unable to say whether actual necrosis was present or whether vagaries in staining accounted for the appearances observed. In the case in which death occurred 3 hours after the burn there was slight separation of the cells of cords of the zona fasciculata to form spaces, but this did not approach the tubule formation described by Rich [25] as characteristic of his cases with infections of various sorts.

Comment

Other organs than those previously mentioned showed changes but these were not subjected to analysis. Congestion and hemorrhage were frequently observed in the earlier deaths. Petechial hemorrhages of the epicardium and endocardium, lungs, stomach and duodenum, and else-

where were noted, as were occasional Curling's ulcers. Extensive hemorrhages into the lungs, with infarct-like areas, occurred in some cases. Congestion of many viscera, such as the spleen and liver, was noted in the cases in which death occurred after a short period of survival. The changes in the lymphoid nodules of the lymph nodes and gastrointestinal tract paralleled the changes in the splenic nodules. No definite or constant alteration was found in the brain. Mention should be made of necrosis and inflammation in the respiratory tract, which occurred from the direct inhalation of flames and fumes.

In the literature on burns, mention is made of the direct effect of heat on the internal organs,[26] with, for example, the production of large vacuoles or bubbles in the liver. Fat embolism has been reported.[10]

Nevertheless, the emphasis should be on the paucity of lesions in the internal organs following fatal burns. If the early changes are interpreted as those of shock and the slightly later ones as those of hemoconcentration, and if the changes in the blood cells and the hemoglobinemia in deep burns are recognized, there are possibly no additional morphologic alterations characteristic of burns other than the changes at the site of the burn. The liver usually shows no necrosis, the changes in the adrenals are possibly those of shock, and karyorrhexis in the lymphoid nodules occurs only in children. There is thus little support for the concept of a powerful burn toxin on the basis of pathologic studies.

Summary and Conclusions

Available for analysis was a series of 96 autopsies in which cutaneous burns or the complications of cutaneous burns were the chief cause of death. The series included 37 cases with death during the first day, 26 cases with death from the second to sixth day inclusive, and 33 cases with death after the first week.

Hepatic necrosis was usually absent, and when present could be explained more reasonably as a result of a complication of the burn, such as infection, then as a direct result of the burn. Necrosis of minimal degree was noted in 4 cases in which no factor but the burn was demonstrated as a cause. In this series of cases tannic acid treatment had not been used to any appreciable extent. In 28 cases from the period before the use of tannic acid there was no case of hepatic damage due to the direct effect of the burns. It was concluded that necrosis of the liver is not a lesion characteristic of burns.

Karyorrhexis of high degree occurred frequently in the lymph nodules of the spleen in those patients less than 17 years of age who died during the first 3 days following burning. Karyorrhexis was absent, or present in minimal degree, in the splenic nodules of those older

than 17 years. Moreover, when those less than 17 years of age survived more than 3 days, karyorrhexis was absent or present in minimal degree. While it could not be proved that the striking karyorrhexis present in the young who died during the first 3 days was not present before burning, it was thought that the karyorrhexis was probably the result of the burn and that it disappeared after the third day because of the digestion of the nuclear particles by phagocytic cells rendering them nonstainable with hematoxylin. It was concluded that changes in the splenic nodules were not fully characteristic lesions of fatal burns, since these changes were not present in adults.

Unequivocal changes in the adrenal and kidney were infrequent. The swelling, congestion, and occasional homorrhage in the adrenals in early deaths were attributed to shock. Hemoglobin casts were noted in the kidneys rarely.

In general, emphasis is placed upon the paucity of histopathologic alterations specific for burns and not attributable to shock, to the rarely occurring hemoglobinemia, or to secondary infection.

The assistance of Dr. Donald deForest Bauer and of Mrs. Margery Prindle is gratefully acknowledged.

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[*Illustrations follow*]

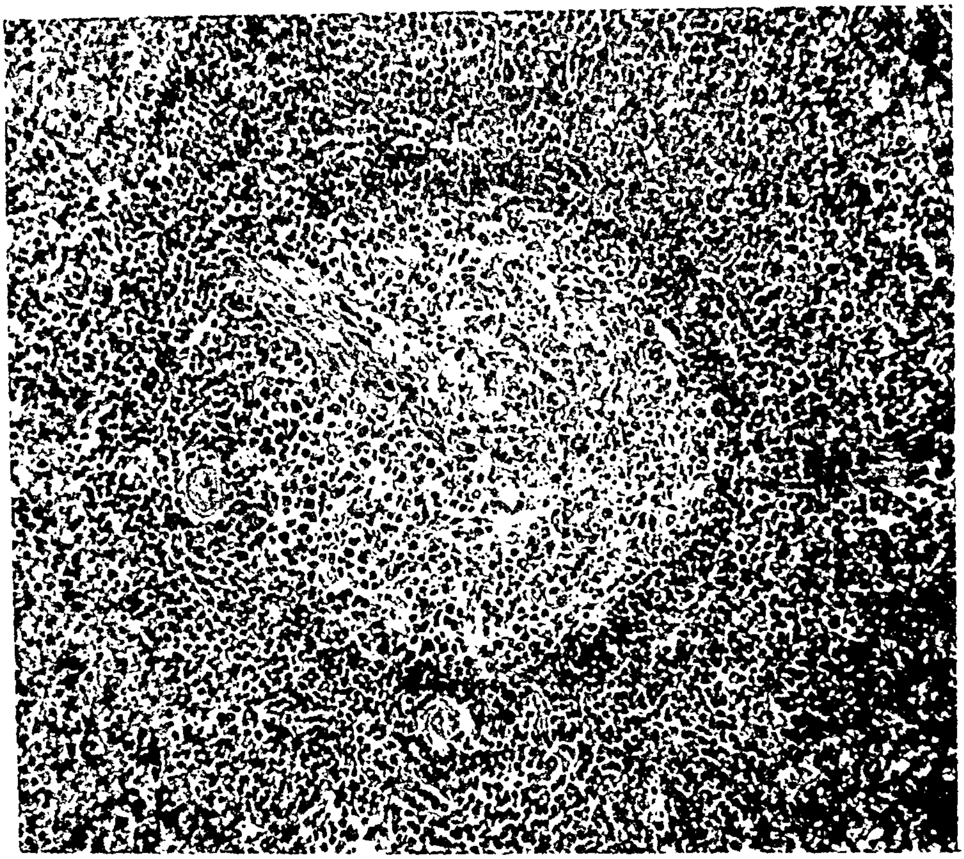
DESCRIPTION OF PLATES

PLATE 122

FIG. 1. Low-power photomicrograph of a splenic nodule from a child, 4 years of age, who survived 3 hours following a burn. The nodule has a large secondary center (pale) with a rim of lymphocytes about it. Hematoxylin and eosin stain. × 50.

FIG. 2. Oil-immersion field from the center of a secondary nodule of the same case as in Figure 1, showing karyorrhexis of 3 plus degree. Most of the nuclear fragments are within phagocytic cells. This case demonstrates that high degrees of karyorrhexis may be encountered as soon as 3 hours after a burn in those less than 17 years of age. Hematoxylin and eosin stain. × 1,458.

1



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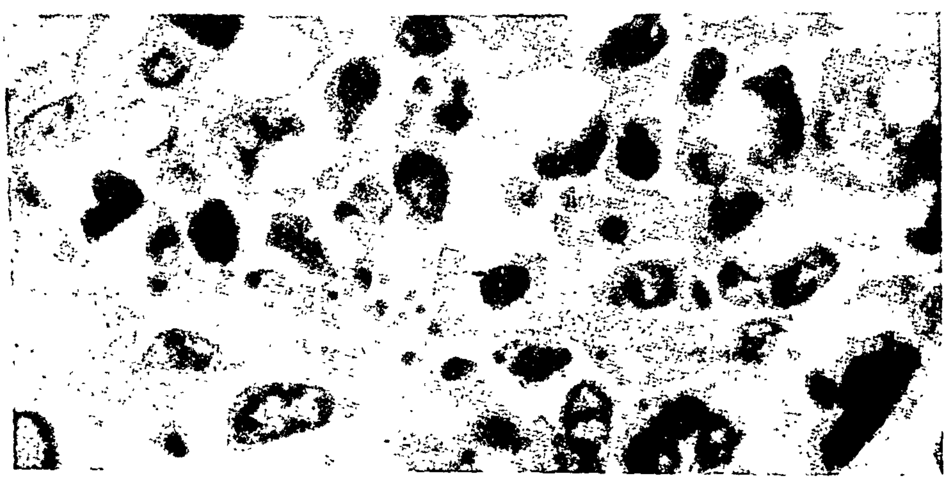

Baker

Internal Lesions in Burns

Plate 123

Fig. 3. Low-power photomicrograph of a splenic nodule from a child, 3 years of age, who survived 20 days following a burn. The nodule contains no secondary center, and typifies the nodules throughout the section. Hematoxylin and eosin stain. × 50.

Fig. 4. Oil-immersion field from the center of the splenic nodule of the same case as in Figure 3. No karyorrhexis is present. This case conforms with the general experience that high degrees of karyorrhexis were not encountered in those patients less than 17 years of age if they survived more than 3 days following the burn. Hematoxylin and eosin stain. × 1,458.

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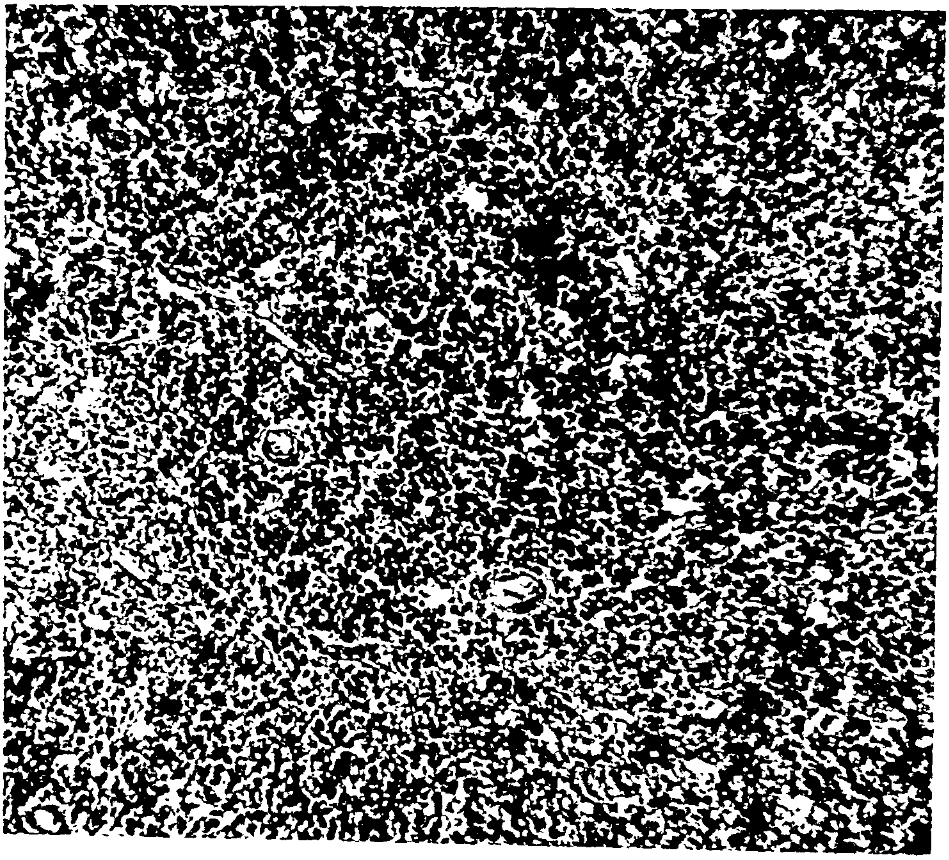

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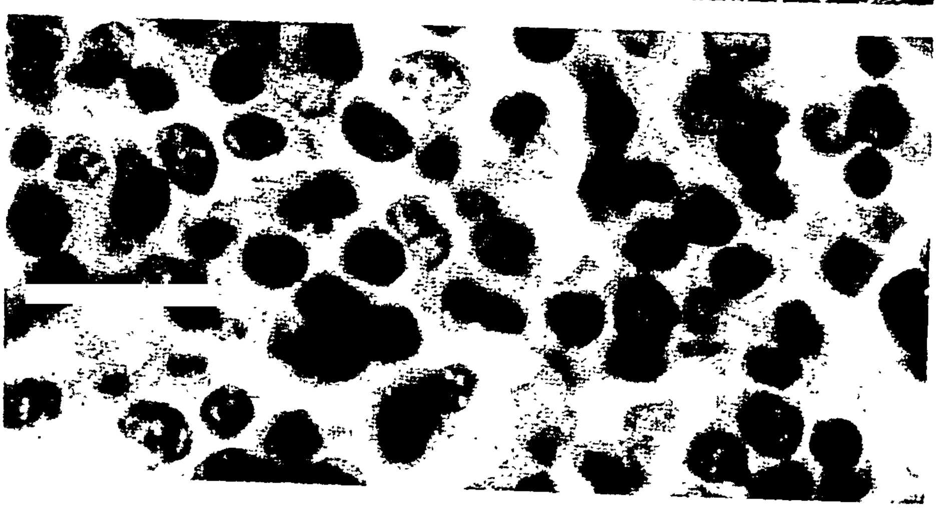

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Internal Lesions in Burns

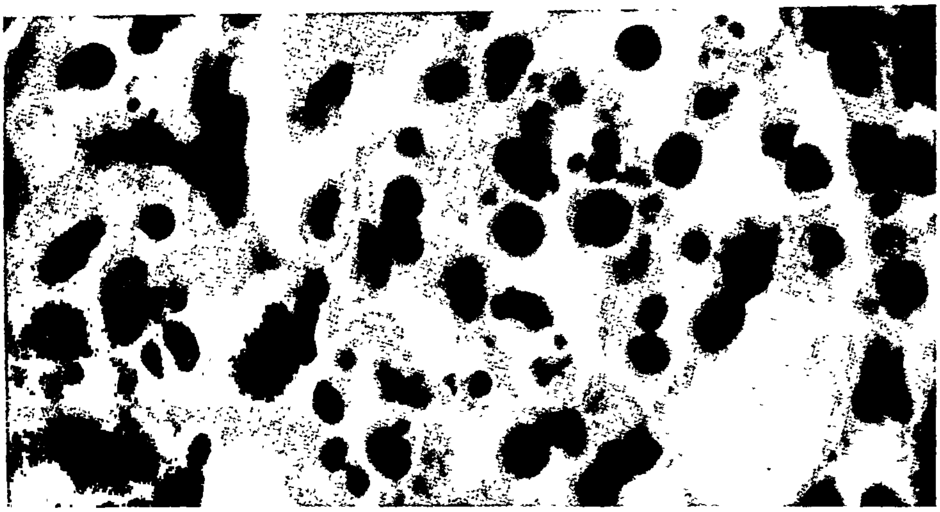
PLATE 124

FIG. 5. Mid-portion of a secondary center of a splenic nodule of a child, 6 years of age, who survived 22 hours following a burn. Karyorrhexis of 3 plus degree is present. As in this example, marked karyorrhexis is usual in young persons dying during the first 3 days following a burn. Hematoxylin and eosin stain. $\times$ 1,458.

FIG. 6. Center of a splenic nodule and edge of a secondary center from a child of 5 years who died 8 days following a burn. Karyorrhexis of 1 plus degree is present. As shown in this case, high degrees of karyorrhexis are not noted in young persons who survive more than 3 days following a burn. The photomicrograph also shows cells with abundant hyaline cytoplasm which may represent material previously in the form of nuclear particles, but which now fails to stain because of intracellular digestion. Hematoxylin and eosin stain. $\times$ 1,458.

FIG. 7. Center of a splenic nodule from an adult, 38 years old, who survived 12 hours following a burn. Karyorrhexis is absent. This conforms with the observation that high degrees of karyorrhexis were not observed in adults. Hematoxylin and eosin stain. $\times$ 1,458.

5



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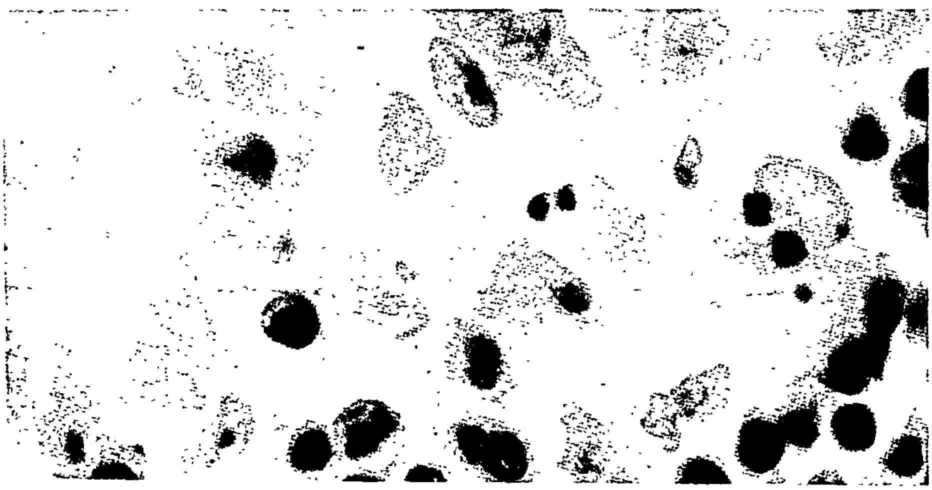

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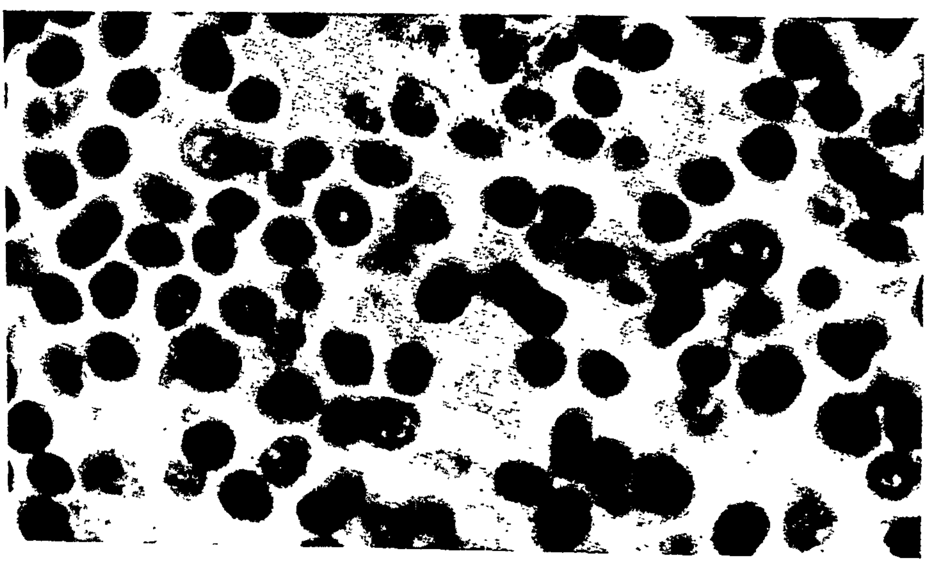

Baker

Internal Lesions in Burns

THE "MASSON BODY" IN RHEUMATIC PNEUMONIA *

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Although the morphologic features of rheumatic fever as it affects the heart, serosal surfaces, and arterioles are well known and agreed upon, the same cannot be said of its manifestations in the lungs. While most observers believe that there is a rheumatic pneumonia, their opinions vary with regard to the exact nature of the lesion. There are (1) those who believe the changes in the lungs are specific,[1-4] (2) those who maintain that they are characteristic but not specific,[5] and (3) those who contend that they are neither characteristic nor specific.[6-8] For the early or acute stages there have been described acute necrotizing and fibrinous alveolitis; hemorrhages; hyaline membranes at the periphery of the alveolar spaces; infiltration of the septa and spaces with monocytes, pigment cells, neutrophils, basophils, plasma cells, and lymphocytes; acute periarteritis with intimal proliferation and hyaline thrombi in the lumina and even Aschoff nodules similar to those found in the myocardium. Later, varying stages of organization and fibrosis have been reported.

Apart from the above-mentioned changes, Masson, Riopelle, and Martin,[9] in 1937, described an inflammatory reaction in the lungs of 13 cases consisting of a peculiar type of cellular granulation tissue in the form of buds which filled the alveolar ducts and spaces. They concluded that rheumatic pulmonary involvement was a specific process. In 1944, Neubuerger, Geever, and Rutledge [10] reported on 8 cases, including both active and quiescent rheumatic fever, in which they found similar granulomatous nodules. They described them as consisting of a central core of pleomorphic cells, some possessing flat spindle-shaped nuclei with scanty neutrophilic cytoplasm and others round, oval, or kidney-shaped nuclei with abundant sharply demarcated cytoplasm. Some of the cells contained golden-brown pigment. The supporting stroma of fibroblastic tissue was loose and fibrillary and contained fibrinous and sometimes mucoid material. Occasionally fibrinous material was collected into a band at the periphery of the granuloma. Capillaries were scanty or entirely absent. Some of the granulomas were covered externally with a single layer of cuboidal cells of "septal" origin. These peculiar formations they termed "Masson bodies" and since similar nodules were not found in 60 control cases of acute pneumonia, passive congestion of the lungs with and without pneumonia, and chronic organizing pneumonia, they concluded that they were

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specific for the rheumatic lung and that they were equivalent to the Aschoff body in the heart.

It is the purpose of this presentation to consider solely the question of specificity of the "Masson body" in rheumatic pneumonitis and to omit entirely a discussion of the already often described and still controversial changes previously referred to.

Method and Material

Microscopic sections of the lungs of 505 cases were studied. They were distributed as follows: uncomplicated rheumatic heart disease, 150; bronchiectasis, 160; tuberculosis, 85; chronic interstitial pneumonitis, 40; organizing pneumonia, 30; abscess, 25; asthma, 12; and periarteritis nodosa, 3. With the exception of 120 surgically removed bronchiectatic lungs, all were obtained from the necropsy file of the Jefferson Medical College Hospital, covering a period of 15 years. Rheumatic heart disease was excluded in the control series by a direct examination of the heart in the cases autopsied and by a careful examination of the histories of the patients whose surgically removed lungs showed the granulomatous bodies. Only those cases were included for which there was at least one satisfactory slide of the lung, and, in the instances of rheumatic heart disease, where there was in addition at least one satisfactory section of the heart. For the most part only file slides were examined. In some cases the original blocks were recut and in a few, where gross specimens were still available, additional material was obtained for study. All sections were stained with hematoxylin and eosin.

Results

Rheumatic Heart Disease

In the 150 cases of rheumatic heart disease, Aschoff nodules were present in the myocardium in 16 and absent in 134. "Masson bodies" were found in the lungs in 3 cases of each group.

Grossly, the lungs of these 6 cases disclosed no characteristic or even constant changes. In 1 case the pleural surfaces were covered with a fibrinous exudate which on closer inspection showed numerous, small, irregularly distributed elevations measuring 1 mm. in height and 3 to 4 mm. in diameter. Two common findings in the lungs proper were an increased resistance upon palpation and, on section, varied degrees of congestion and edema. Irregular areas of pneumonic infiltration were also observed, but they did not differ in any way from those of ordinary pneumonia.

Microscopically, the outstanding feature was the patchy distribution of the "Masson bodies." Their histologic structure varied somewhat but the general pattern appeared to be the same in all cases. The nodules might conveniently be divided into two groups, early and late, although it is to be understood that there was no sharp line between the two, but rather a gradation from one to the other. The early lesions seemed to arise either in the septa proper or within the alveolar ducts or spaces. Those of the former origin were by far the less frequent. Each consisted of a round or fusiform, unilateral or bilateral bulging of the septum caused at first by a fibrinoid necrosis of the wall and later by a gradual replacement with loose fibrillary connective tissue and a mixture of various types of inflammatory cells. As the process progressed the nodules became more circumscribed but still retained their septal connections. Sometimes it was impossible to tell whether the lesion started in the septum or without. Ordinarily, however, the latter origin was quite evident and constituted the more frequent site.

In 2 patients who were 3½ and 7 years old, and who apparently died in the initial attack, the lesions in the alveolar spaces and ducts were very early and in various stages of development. They consisted essentially of a mixture of fibrin, monocytes, and very occasionally polymorphonuclear leukocytes, plasma cells, and pigment cells. Usually fibrin predominated. While some of these arrangements were unattached to the alveolar septa, most of them were united to the latter by a broad base or one or more slender pedicles. As the lesions progressed the fibrin became less deeply stained and was gradually replaced with loose edematous connective tissue. Externally many of the bodies were partially or almost completely covered with a single layer of flat or low cuboidal cells. Occasionally there was a condensation of the fibrinous and fibroblastic material at the periphery of the nodule while the center was filled with monocytes, pigment cells, and a few plasma cells.

The distribution of the nodules in the late stage, as in the early, was also patchy. In all sections where "Masson bodies" were found, however, there were also present a few irregular plugs of organizing or organized exudate. These were essentially similar in structure and age to the "Masson bodies," and differed only in their external configuration. Again, as in the early lesion, most of the granulomas were attached to the ductal and septal walls at one or more points by narrow or broad pedicles. Sometimes, however, they appeared to be a direct expansion of the septum or alveolar duct wall itself. They were all sharply circumscribed and round, oval, or less frequently fusiform. Often there was an external covering of a single row of flat or cuboidal cells. Most

of them contained neither blood vessels nor erythrocytes. They were easily divided into four types, namely: (1) fibrillary, consisting of fibrin mixed with edematous fibroblasts and often arranged more or less concentrically like the layers of an onion (Fig. 1); (2) fibrous, consisting entirely of dense fibrous tissue (Fig. 2); (3) granulomatous, consisting of a few spindle cells with spindle-shaped nuclei; large, round or oblong cells with abundant pink-staining cytoplasm and large, round, evenly stained nuclei; phagocytes containing ingested brown pigment, and small round cells with almost imperceptible cytoplasm (Fig. 3); (4) mixed forms of the above, consisting of the various elements just mentioned, either irregularly scattered throughout the nodules, or separated into a central core of inflammatory cells surrounded peripherally by a band of fibrinous material or fibrous tissue. Ordinarily all types of granulomas were found in the same patient and even in the same slide.

Bronchiectasis

In 160 cases of bronchiectasis there were 26 that showed "Masson bodies." These were in all respects similar to and frequently identical with, those found in rheumatic lungs. Their distribution was patchy and only in a few instances were there more than two or three in a single field (Fig. 4). Some appeared to have originated in the septa while others seemed to have started in the spaces and to have become attached to the adjoining walls by broad or thin pedicles. Structurally, as in the rheumatic lungs, they were either (1) fibrillary (Fig. 5), (2) fibrous (Fig. 6), (3) granulomatous (Fig. 7), or (4) mixed. The granulomatous type differed slightly from those similarly designated in the rheumatic lungs. Cellularity was usually more marked; plasma cells were more abundant; phagocytic cells contained ingested lipoid material instead of pigment, and, finally, the surrounding alveolar septa were broader and densely infiltrated with a mixture of similar cells. The remaining three types of bodies in this group were so similar to those in the rheumatic lungs that in some instances they could practically be superimposed. Organized exudate similar in composition to the "Masson bodies" was also seen scattered throughout.

Tuberculosis

In 85 cases of pulmonary tuberculosis there were 7 that showed "Masson bodies." As in the rheumatic and bronchiectatic lungs, they included all types previously referred to. Some were almost mirror images of those found in bronchiectasis and rheumatic heart disease (Fig. 8). All cases that showed "Masson bodies" also showed plugs of exudate in varying stages of organization.

Chronic Interstitial Pneumonitis

In 40 cases of chronic interstitial pneumonitis there was only 1 case that showed a few "Masson bodies" of the granulomatous type (Fig. 9). Here, however, as in the granulomatous type seen in bronchiectasis, the structure was somewhat different from that of the third type seen in the rheumatic lungs. The cells were mostly plasma cells and the septa from which they sprang were greatly thickened as a result of infiltration with similar cells. None of these 40 cases showed organizing pneumonia.

Organizing Pneumonia

In 30 cases of organizing pneumonia there were 14 with "Masson bodies." In all of these the pneumatic exudate was in various stages of organization, and was the most conspicuous change. There were, however, two other outstanding features in this group of cases. Firstly, the bodies were practically all of the fibrillary or fibrous types; and, secondly, capillaries and erythrocytes were practically non-existent. In some cases there was unmistakable evidence that the organized exudate in the spaces became attached to the alveolar wall (Fig. 10). In others the nodules were already rounded off and covered with cuboidal or attenuated cells. Golden-brown or black pigment was frequently seen within many of the bodies of the fibrous type.

Pulmonary Abscess

In 25 cases with pulmonary abscesses there were 5 that showed "Masson bodies." These were of the granulomatous, fibrous, and mixed types. Several of the mixed type had a broad peripheral band of fibrous tissue which also formed the main part of the pedicle (Fig. 11). The central portion was filled with spindle cells, plasma cells, small round cells, and phagocytes with and without pigment. There were no capillaries. All cases showing "Masson bodies" also disclosed alveolar exudate in similar stages of organization.

Asthma

Of 12 cases of asthma none showed "Masson bodies" and none showed organizing or organized pneumonia.

Periarteritis Nodosa

In 3 cases of periarteritis nodosa there was 1 that showed a "Masson body." It appeared to be a bilateral bulging of a septum (Fig. 12). It was composed of loose edematous and fibrillary connective tissue and contained scattered plasma cells and pigment cells. One surface was covered with low cuboidal cells. Nearby there was an organizing infarct

and the neighboring alveolar spaces contained scattered plugs of organizing exudate.

Discussion

Although Neubuerger, Geever, and Rutledge [10] "discovered the 'Masson body' in a few instances wherein no previous rheumatic history was elicited," they nevertheless concluded that despite a few exceptions of this type "the 'Masson body' is a fairly specific granuloma." In a larger and somewhat different series of control material we have found the "Masson body" in a high proportion of cases of organizing pneumonia, pulmonary abscess, bronchiectasis, and tuberculosis, and are therefore of the opinion that such granulomas are not specific for rheumatic pneumonia but are found in a wide variety of pulmonary disorders. That the control cases were not also affected with rheumatic fever was made reasonably certain in the autopsied group by a direct examination of the heart, and in the surgically removed lungs, showing "Masson bodies," by a careful examination of the case histories. Since most of the positive cases were obtained by an examination of consecutive file slides, with frequently only one slide available from a case, we are also certain that had more sections from different areas of the lungs been examined, the number of positives would have been much higher.

The relation of the "Masson body" to organizing or organized pneumonia is of more than passing interest. Although Neubuerger, Geever, and Rutledge [10] gave special attention to the differential diagnosis between the two, some of the differences mentioned were not too apparent in our material. It is noteworthy that all cases in this series, both rheumatic and non-rheumatic, that showed "Masson bodies" also disclosed in the same sections a few scattered plugs of unmistakable organized pneumonic exudate. Furthermore, the structural composition of both the "Masson body" and the organized exudate was generally the same. When the former was composed of fibrillary connective tissue so was the latter. When one possessed a few pigment or foam cells so did the other. When there was an external covering of cuboidal cells on the "Masson body," so were there similar cells covering the organized exudate. Capillaries and extravasated erythrocytes were practically nonexistent in either structure. For these reasons we believe that in most cases the "Masson body" is organized nonspecific exudate which has assumed a polypoid, round, or oval shape and become attached to the septal or ductal wall as the inevitable result of organization. In fact, in the acute rheumatic pneumonia described in this report, all graduations from the presence of a mass of fibrinous material in the alveoli and ducts to the fully formed "Masson body" have been traced.

On the other hand it should again be pointed out that occasionally these granulomas do originate in the septal wall itself. This was seen definitely in some of the cases of rheumatic fever, in bronchiectasis, and in chronic interstitial pneumonia.

The cases of asthma and periarteritis nodosa were included in this report because it was thought that if the "Masson body" is specific for rheumatic fever, which is probably an allergic disease, it should also be found in asthma and periarteritis nodosa. Unfortunately the number of cases at our disposal was too small from which to draw definite conclusions. It might, however, be of interest to note that, although many histologic sections of the lungs of these cases were examined, only one "Masson body" was found in one case of periarteritis nodosa, and nearby there were an organizing infarct and a few irregular plugs of organized pneumonic exudate.

Summary and Conclusions

In a study of the lungs of 505 cases, "Masson bodies" were found in approximately 46 per cent of those with organizing pneumonia; 20 per cent, with pulmonary abscess; 16 per cent, with bronchiectasis; 8 per cent, with pulmonary tuberculosis; 4 per cent, with rheumatic heart disease; 2 per cent, with chronic interstitial pneumonitis; 1 of 3 cases of periarteritis nodosa; and in none of 12 cases of asthma. In all these cases there were also a few nearby plugs of organized exudate which did not differ from the "Masson body" in their structural composition, but only in their external configuration. We are therefore of the opinion that most "Masson bodies" are organized intra-alveolar and intraductal exudate, and that only a few originate in the septal and ductal walls. Since these granulomas are found in a wide variety of pulmonary disorders, they are not specific for rheumatic pneumonitis.

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DESCRIPTION OF PLATES

PLATE 125

FIG. 1. From a case of rheumatic fever, showing a "Masson body" of the fibrillary type. It has two pedicles and is composed of fibrin and loose fibrillary connective tissue with very few central plasma cells. There is a single layer of flat cells covering a portion of the periphery. Hematoxylin and eosin stain. × 200.

FIG. 2. From a case of rheumatic fever, showing a "Masson body" of the fibrous type attached to a septum at one point. It is composed of dense fibrous tissue and is covered with a single layer of flat cells. Hematoxylin and eosin stain. × 200.

FIG. 3. From a case of rheumatic fever, showing a "Masson body" of the granulomatous type. It has a broad base and is composed of spindle cells, round cells with abundant and distinct cytoplasm, round cells with almost imperceptible cytoplasm, and phagocytes containing brown pigment. There are a few peripheral cuboidal cells at the tip. Hematoxylin and eosin stain. × 200.

FIG. 4. From a case of bronchiectasis, showing seven "Masson bodies" of the fibrillary type. Hematoxylin and eosin stain. × 50.

FIG. 5. One of the bodies in Figure 4 at a higher magnification. It is composed of loose fibrillary connective tissue somewhat concentrically arranged. The periphery is partially covered with a single layer of flat cells. Hematoxylin and eosin stain. × 200.

FIG. 6. From a case of bronchiectasis, showing a "Masson body" of the fibrous type. It is attached by a thin and broad pedicle and is composed of dense fibrous tissue. It has an incomplete external covering of flat cells. Hematoxylin and eosin stain. × 200.

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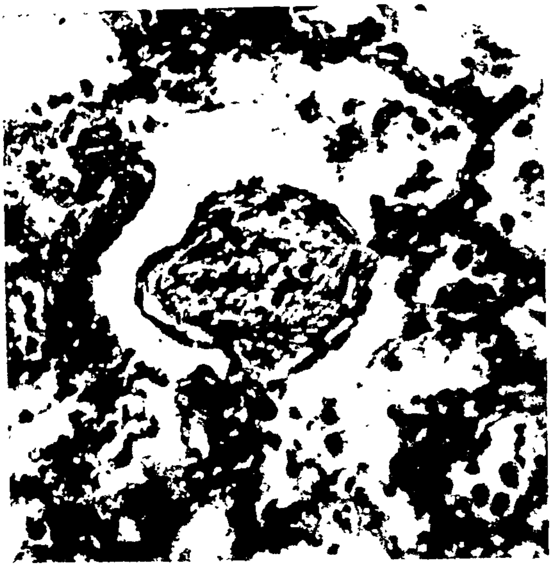

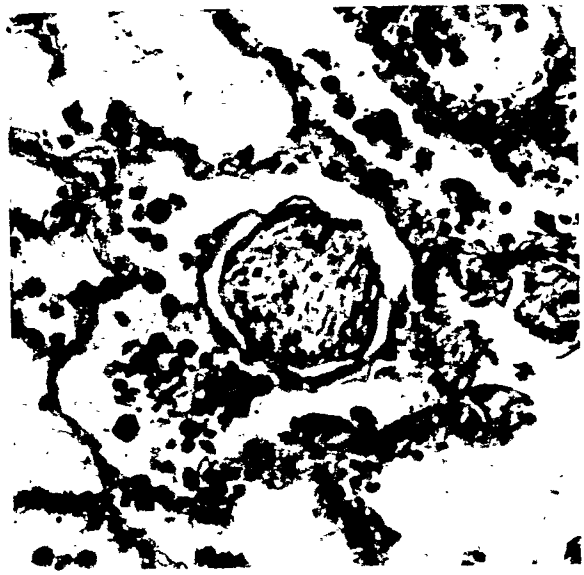

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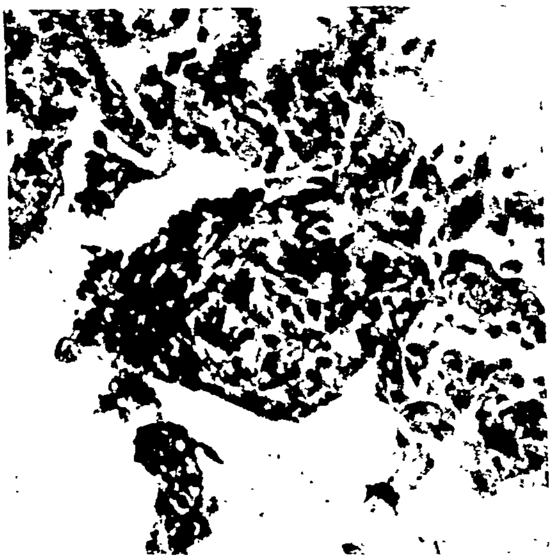

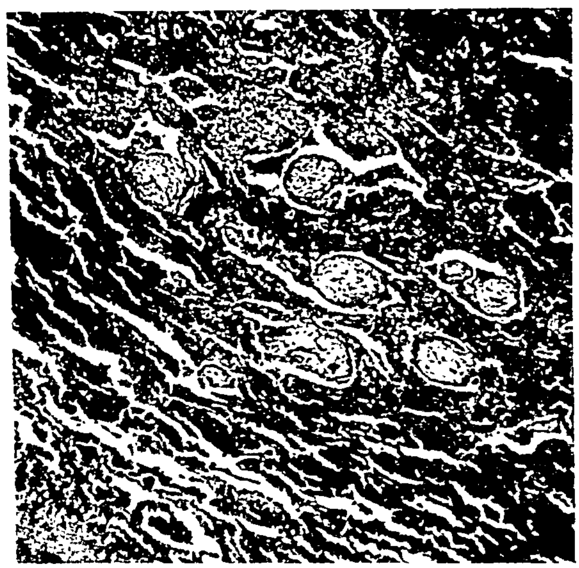

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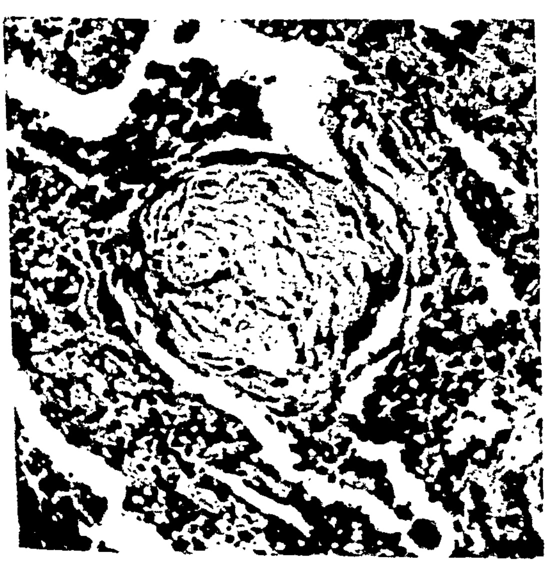

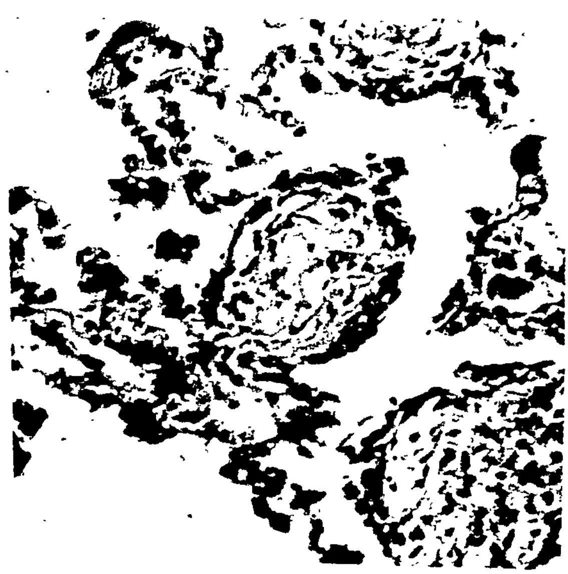

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The "Masson Body" in Rheumatic Pneumonia

PLATE 126

FIG. 7. From a case of bronchiectasis, showing a "Masson body" of the granulomatous type. It is attached by a thin pedicle and is composed of plasma cells, lymphocytes, a few polymorphonuclear leukocytes, many phagocytes containing ingested lipoid material, and two tiny capillaries. Externally there is a single row of cuboidal cells. Hematoxylin and eosin stain. × 200.

FIG. 8. From a case of pulmonary tuberculosis, showing a "Masson body" composed of concentrically arranged connective tissue and a central collection of plasma cells, lymphocytes, and phagocytes containing brown pigment. Hematoxylin and eosin stain. × 200.

FIG. 9. From a case of chronic interstitial pneumonitis, showing a "Masson body" of the granulomatous type. It is attached by a thin pedicle and is composed almost entirely of plasma cells and fewer lymphocytes in a background of loose connective tissue. There is an external covering of a single layer of cuboidal cells. Hematoxylin and eosin stain. × 200.

FIG. 10. From a case of organizing pneumonia, showing a transition stage between a plug of organized exudate and a "Masson body." It is composed of a mass of dense fibrous tissue united to the septa at two points. Centrally there are present a few plasma cells and phagocytes laden with brown pigment. Blood vessels are absent. Hematoxylin and eosin stain. × 200.

FIG. 11. From a case of pulmonary abscess, showing a "Masson body" of the mixed type. It consists of an outer zone and pedicle of dense fibrous and loose fibrillary connective tissue. Centrally there are spindle cells, plasma cells, lymphocytes, and phagocytes with and without brown pigment. Hematoxylin and eosin stain. × 200.

FIG. 12. From a case of periarteritis nodosa, showing a "Masson body" that seems to be arising as a bilateral swelling of a septum. It is composed of loose fibrillary connective tissue in which there are a few plasma cells and phagocytes containing brown pigment. One border is partially covered with a single layer of cuboidal cells. Hematoxylin and eosin stain. × 200.

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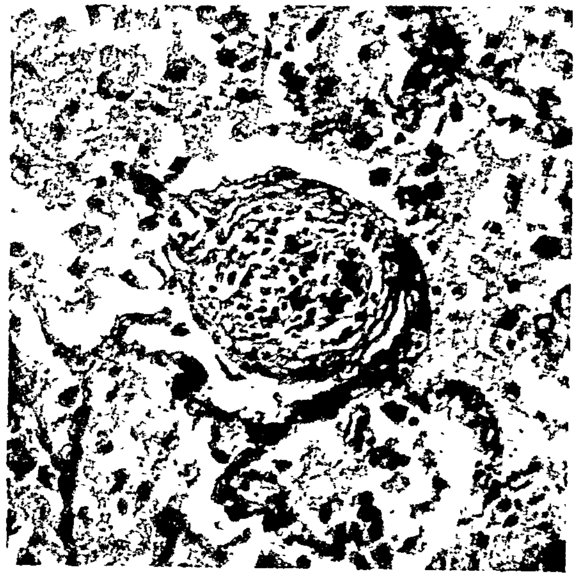

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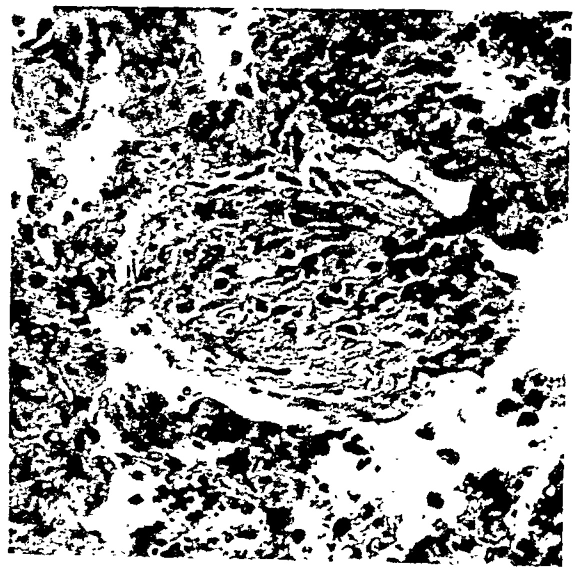

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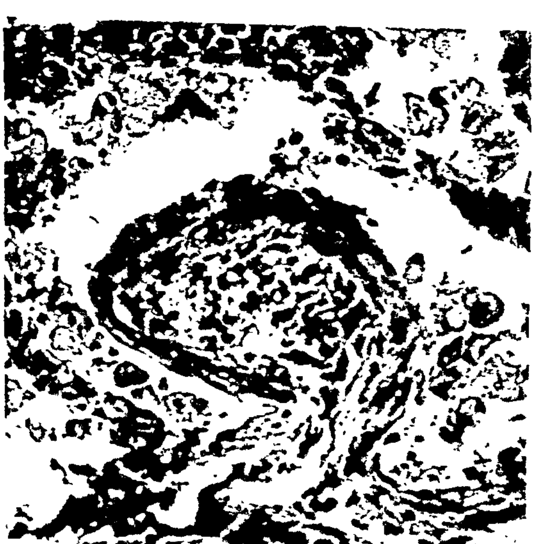

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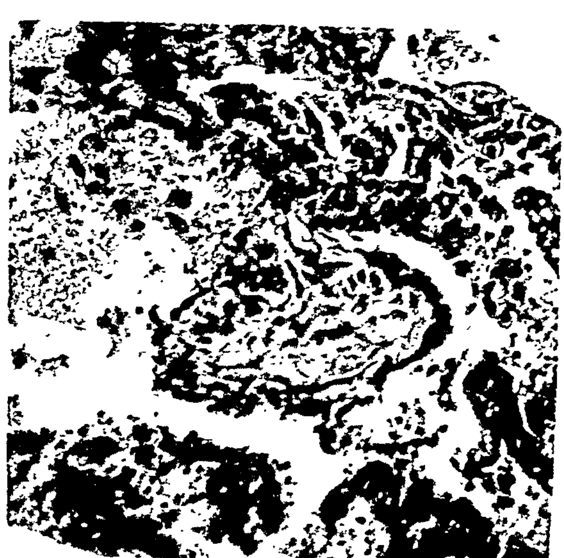

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The "Masson Body" in Rheumatic Pneumonia

THE REACTION OF THE RETICULO-ENDOTHELIAL SYSTEM IN EXPERIMENTAL BRUCELLOSIS OF DOGS *

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The experimental studies reported in this paper relate to the general problem of the reaction of mammalian tissues to infection by *Brucella suis*, but with special emphasis upon the response of the cells belonging to the reticulo-endothelial system. This investigation was made as a part of a more comprehensive program, the main purpose of which is to test the possibility of an etiological relation between brucella infection in man and Hodgkin's disease. In previously published papers we have reported studies on experimental brucellosis in a variety of animals, including guinea-pigs,[1] hogs,[2, 3] dogs,[3] and on naturally acquired brucellosis in man,[4, 5] all of which relate to this problem. In all of the experimental studies the strains of brucella employed were derived from cases of typical Hodgkin's disease; a strain of brucella derived from a naturally infected hog was used for the production of comparative infection. In this paper are described in some detail the pathological anatomical findings in a group of dogs in which infection by a strain of *Br. suis* had been maintained for as long as 487 days by repeated inoculations. The bacteriological and immunological observations made in this experiment will be dealt with only in brief; they have been reported in full in a previous communication.[3]

The literature dealing with brucellosis in dogs, both the naturally acquired and the experimental disease, is quite limited; this has been reviewed in one of our earlier communications.[3] At this time only a brief note relating to the previously reported pathological anatomical findings is pertinent. It has been observed that the development of anatomical lesions in both the naturally acquired and the experimental infection is quite unusual; even so, in the hands of practically all experimenters, there appears to have been no great difficulty in establishing infection in the dog as indicated by a significant rise in the agglutination titer for brucella and the recovery of the organisms either from the tissues at autopsy or from the blood during life. The rare instances in which anatomical lesions have been described in brucellosis in dogs and the lesions described were: (a) enlargement and suppuration of the testis (Plantz and Huddleson,[6] Davis [7]), (b) multiple yellowish nodules in the kidney (Thomsen [8]), (c) enlargement of the reticulo-

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endothelial tissues, especially the lymph nodes and the spleen (van der Hoeden [9]), (d) focal, histiocytic, tubercle-like reactions in the liver, spleen, kidneys, and lungs (Feldman, Bollman, and Olson [10]).

Of the descriptions just mentioned, that of Feldman, Bollman, and Olson [10] is most informative. The lesions they describe were found in dogs experimentally infected with organisms obtained from swine and bovines. The dogs were inoculated by mouth and intravenously, only one inoculation being made. The total duration of the experiment was 185 days. The histological descriptions of the lesion by Feldman, Bollman, and Olson, unlike those of the other contributors, are quite comprehensive, and they indicate that the basic reaction in the dog is that which involves a response of the cells of the reticulo-endothelial system. In all of the studies that have been made, the conclusion has been reached that the dog has but little susceptibility to injury by brucella and that whatever lesions may be produced are minimal and transient. In view of this conclusion and in view of our primary objective, that is, to produce in the dog, if possible, a disease resembling Hodgkin's disease in man, it was decided to use, in our experiments, procedures that were significantly different from those utilized by preceding workers. Accordingly, in the experiments, reports of which follow, repeated inoculations were made by different routes and at considerable intervals of time, and the experiments were continued for as long as 300 days in excess of the experimental period in the investigations of Feldman, Bollman, and Olson.

Experiments

Nine healthy dogs were employed in our experiment. Seven of these were used for a study of infection by a strain of brucella obtained from a typical case of Hodgkin's disease in an adolescent boy who had died of the disease after an illness of over 5 years. The strain of brucella recovered from this case (herein referred to as the Brody strain) was of the suis variety. Previously it had been shown to be pathogenic for the guinea-pig, and it had been used in other experiments carried out on the hog; this organism was known to be virulent for the guinea-pig at the time the experiments on the dog were begun. Two of the dogs were used for a comparative study of infection by a strain of *Br. suis* which had been recovered from a natural infection in a hog (herein referred to as strain ABF 36). This organism was known to be pathogenic for the hog and the guinea-pig at the time the dog experiments were begun.

Preliminary studies involving the determination of the agglutination titer for brucella 456, the opsonocytophagic index, and cultures of the blood for brucella were carried out on all of the experimental animals.

Each animal was kept in its own cage from which it was never re-

moved except for inoculation. All animals were kept in a room in which there were also other animals infected by the organisms being employed in the experiment. Special care was exercised in the handling of all animals in this group to prevent cross infection.

Four of the animals inoculated with the Brody strain of brucella received these organisms intravenously. Three of the animals receiving the Brody strain were inoculated intraperitoneally. One of the animals receiving the ABF 36 strain of brucella was inoculated intraperitoneally

TABLE I

Experimental Brucellosis in Dogs: Experimental Data

Dog	Length of experiment in days	Organism and route	Injections	Blood cultures	Positive blood cultures	Days before death of: Last injection	Days before death of: Last positive blood culture	Organ cultures at autopsy: Nodes	Spleen	Liver	Kidney	Testis	Lung
I	198 died	Brody I. V.	21	27	12	14	7	+	+	+	+	−	−
II	487 killed	Brody I. V.	35	42	18	225	233	+	−	−	+	−	o
III	461 killed	Brody I. P.	39	41	4	143	270	−	−	−	−	o	o
IV	261 died	Brody I. V.	28	34	23	7	7	o	+	+	+	+	−
V	38 died	Brody I. V.	4	5	3	8	1	+	+	o	o	o	o
VI	454 killed	Brody I. P.	39	43	o	136		−	−	−	−	−	o
VII	398 killed	Brody I. P.	33	34	8	132	231	−	−	−	−	o	−
IX	186 killed	ABF 36 I. P.	3	5	1	152	152	+	−	−	−	−	o
X	216 killed	ABF 36 I. V.	3	5	3	182	101	+	−	−	−	o	o

and the other intravenously. The inoculations were given repeatedly, usually at intervals of about 1 week. In certain instances the inoculations were given in two series, the second series being started after the animal had been allowed to recover sufficiently from the preceding inoculations to assure prolongation of the experiment.

All of the dogs were bled from the jugular vein at frequent intervals and always preceding inoculation. Blood cultures were made and brucella agglutination titers and opsonocytophagic indices were determined at each bleeding. The essential data relating to the experiment are recorded in Table I. The bacteriological and immunological observations made during the course of this experiment are the subject

of a previous communication in which all of the details may be found.[3] Protocols in summary for each of the experimental animals follow.

Dog I. Summary of Protocol

When the experiment was begun, on August 9, 1940, dog I, a male mongrel, weighed 9.3 kg. It was fed on a stock diet. Preliminary blood studies showed: opsonocytophagic index, 37; agglutination for brucella 456, negative. The animal died 198 days after the first injection.

Dog I received 21 intravenous injections of brucella (Brody strain), each inoculation consisting of 10 billion organisms. The first 14 injections were given at weekly intervals; from that time injections were given irregularly, at an average of bi-monthly intervals. The last injection was given 14 days before death.

Temperature taken every 3 days for the first 2 months ranged between 37.8° and 40° C. Opsonocytophagic index varied widely during the experiment. From an initial 37 it rose gradually to 91.5, then varied between 56 and 80.5. There was no correlation of this index with the course of the disease. Agglutination of brucella 456, initially negative, rose to 1:5120 at the end of the first week and varied from 1:2560 to 1:20480 during the experiment. Brucella was recovered from the blood stream after the first injection, and a total of 12 times in 27 weekly cultures. The positive results were obtained from cultures made 1 week after injections; blood cultures at intervals of 2 or more weeks following inoculations were always negative.

During the first 11 weeks of the experiment the dog appeared well. Then, with continued weekly injections he developed anorexia, rapidly lost weight, and at 14 weeks appeared moribund. At this time multiple shallow ulcers appeared in his mouth. These did not resemble the lesions of black tongue and did not show a characteristic response to adequate parenteral doses of nicotinic acid. Brucella was isolated from the ulcers 15 days after the last previous injection of organisms. When inoculations were discontinued the ulcers slowly healed and the general condition of the animal improved. When injections were started again and given at shorter intervals, the dog seemed to hold his own for a while, but then again developed anorexia, became very weak and cachectic, and at death presented a picture of extreme emaciation. No enlargement of the peripheral lymph nodes was observed during the experiment.

Autopsy was performed immediately after death. Findings are summarized in Table II.

Dog II. Summary of Protocol

When the experiment was begun, on August 9, 1940, dog II, a male mongrel, weighed 10 kg. It was fed on a stock diet. Preliminary blood studies showed: opsonocytophagic index, 50; agglutination for brucella 456, negative. The animal was killed 487 days after the first injection.

Dog II received 35 intravenous injections of brucella (Brody strain), each inoculation consisting of 10 billion organisms. The inoculations were given at almost weekly intervals. The animal was then kept for the duration of the experiment without further injections. The last inoculation was given 225 days before death.

Temperature taken every 3 days during the first 2 months ranged between 38.4° and 39.8° C. Opsonocytophagic index varied widely during the experiment, from an initial 50 to a low of 35 and a high of 87. There was no correlation of this index with the course of the disease. Agglutination of brucella 456, initially negative, rose to 1:5120 at the end of the first week, and fluctuated from 1:1280 to 1:20480 during the experiment. Brucella was recovered from the blood stream

after the first inoculation, and a total of 18 times in 42 cultures. All of the positive cultures were obtained 1 week after inoculations; blood cultures at intervals of 2 or more weeks following injections were always negative.

During the first 30 weeks of the experiment the dog appeared well except for periods of anorexia and mild weight loss. He then developed signs of illness, including pronounced lassitude, anorexia, weakness, and conspicuous weight loss. After 37 weeks it appeared that the animal would not survive further injections, and the inoculations were discontinued. The dog gradually recovered and remained well for the rest of the experiment. No peripheral lymph node enlargement was noted during the period of observation.

Dog was killed by intracardiac air, and autopsy was performed immediately after death. Findings are summarized in Table II.

Dog III. Summary of Protocol

When the experiment was begun, on August 9, 1940, dog III, a female mongrel, weighed 10 kg. It was fed on a stock diet. Preliminary blood studies showed: opsonocytophagic index, 32; agglutination for brucella 456, negative. The animal was killed 461 days after the first injection.

Dog III received 39 intraperitoneal injections of brucella (Brody strain), each inoculation consisting of 10 billion organisms. These were given at weekly intervals except for the last few injections, which were given at bimonthly intervals. The last inoculation was given 143 days before death.

Temperature taken every 3 days for the first 2 months ranged between 37.9° and 39.1° C. Opsonocytophagic index fluctuated widely during the experiment. From an initial level of 32 it varied between 25.5 and 83. There was no correlation of this index with the course of the disease. Agglutination of brucella 456, initially negative, rose to 1:5120 after 1 week, and thereafter fluctuated between 1:1280 and 1:20480. Brucella was isolated from the blood 1 week after the first and the second inoculations, but was recovered only on two subsequent occasions in a total of 41 cultures. No positive cultures were obtained later than 1 week following injection.

During the course of the experiment the dog showed no definite signs of illness. No signs of peritonitis were evident. No peripheral lymph node enlargement could be detected during the experiment.

Dog was killed by intracardiac ether (5 cc.). Autopsy was performed immediately after death. Findings are summarized in Table II.

Dog IV. Summary of Protocol

When the experiment was begun, on August 9, 1940, dog IV, a male mongrel, weighed 8.5 kg. It was fed on a stock diet. Preliminary blood studies showed: opsonocytophagic index, 28; agglutination for brucella 456, negative. The animal died 261 days after the first injection.

Dog IV received 28 intravenous injections of brucella (Brody strain), each inoculation consisting of 10 billion organisms. The first 19 injections were at weekly intervals; thereafter the inoculations were given irregularly, at an average of bimonthly intervals. The last injection was 7 days before death.

Temperature taken every 3 days for the first 2 months ranged between 38° and 39.5° C. Opsonocytophagic index, initially 28, varied between 44.5 and 90 during the experiment, and did not appear to be correlated with the course of the disease. Agglutination of brucella 456, initially negative, rose to 1:5120 at the end of 1 week and fluctuated between 1:1280 and 1:20480 during the experiment. Brucella was isolated from the blood stream 1 week after the first injection, and a total of 16 times in 19 weekly cultures made during the period of weekly injec-

tions. Of 15 cultures made thereafter, 7 were positive. All positive results were obtained from cultures taken 1 week after injections; blood cultures taken at intervals of 2 or more weeks after inoculations were always negative.

During the first 12 weeks of the experiment the dog appeared well. He then developed anorexia, lassitude, weakness, and lost weight; the weekly injections were discontinued after 18 weeks because it appeared that the animal would not survive much longer. The animal improved, and injections were resumed at bimonthly intervals. It appeared that these were being withstood very well, and weekly injections were resumed, but after the fifth of these the dog showed a rapid decline in weight, appetite, and strength, and died presenting a picture of cachexia. No peripheral lymph node enlargement was noted during the experiment.

Autopsy was performed 3 hours after death. Findings are summarized in Table II.

Dog V. Summary of Protocol

When the experiment was begun. on August 9, 1940, dog V, a male mongrel, weighed 9.0 kg. It was fed on a stock diet. Preliminary blood studies showed: opsonocytophagic index, 20; agglutination for brucella 456, negative. The animal died 38 days after the first injection.

Dog V received 4 intravenous inoculations of brucella (Brody strain), each injection consisting of 10 billion organisms, at about weekly intervals. The last injection was 8 days before death.

Temperature taken every 3 days ranged between 38° and 40.3° C. Opsonocytophagic index, initially 20, varied between 21.5 and 51.5. Agglutination of brucella 456, initially negative, rose to 1:5120 after 1 week, and ranged between 1:2560 and 1:10240. Brucella was recovered from the blood after the first inoculation, and a total of 3 times in 5 subsequent cultures; all positive cultures were obtained at intervals of 1 week after inoculations.

The animal appeared well until the 38th day when there was sudden onset of a shock-like condition and rapid death. Autopsy performed 1 hour after death revealed the stomach to be filled with impacted shavings. Other findings are summarized in Table II.

Dog VI. Summary of Protocol

When the experiment was begun, on August 9, 1940, dog VI, a male mongrel, weighed 8.4 kg. It was fed on a stock diet. Preliminary blood studies showed: opsonocytophagic index, 7; agglutination for brucella 456, negative. The animal was killed 454 days after the first injection.

Dog VI received 39 intraperitoneal injections of brucella (Brody strain), each inoculation consisting of 10 billion organisms. The injections were given at almost weekly intervals over a period of 47 weeks. The last inoculation was 136 days before death.

Temperature taken every 3 days for 2 months ranged between 37.8° and 39° C. The opsonocytophagic index, initially 7, fluctuated widely during the first 6 weeks, then ranged between 53 and 86.5. Agglutination of brucella 456, initially negative, rose to 1:5120 after 1 week, and fluctuated between 1:1280 and 1:20480 during the experiment. Brucella was never recovered from the blood stream in 43 attempts: 38 of the cultures were made at intervals of 1 week following injection; 3 were made at longer intervals; and 2 were taken 1 day following inoculation.

During the entire experiment the dog remained well. At no time was there evidence of peritonitis, and no enlargement of the peripheral nodes occurred.

Dog was killed with intracardiac ether (5 cc.); autopsy was performed immediately afterwards. Findings are summarized in Table II.

Dog VII. Summary of Protocol

When the experiment was begun, on September 30, 1940, dog VII, a male mongrel, weighed 8.6 kg. It was fed on a stock diet. Preliminary blood studies showed: opsonocytophagic index, 7; agglutination for brucella 456, negative; blood culture, negative. The animal was killed 398 days after the first injection.

Dog VII received 33 intraperitoneal injections of brucella (Brody strain), each inoculation consisting of 10 billion organisms. These injections were given at almost weekly intervals over a period of 38 weeks. The last injection was 132 days before death.

The opsonocytophagic index rose rapidly from 7 to a level ranging from 41 to 84 during the experiment. Agglutination of brucella 456, initially negative, rose to 1:5120 and varied between 1:1260 and 1:20480 during the experiment. Brucella was isolated from the blood stream 8 times in 34 cultures. The first positive culture was obtained after the eighth inoculation. All positive cultures were obtained after intervals of 1 week following injection.

Throughout the experiment the dog remained well. At no time was there evidence of peritonitis, nor any enlargement of the peripheral lymph nodes.

Dog was killed with intracardiac ether (1 cc.); autopsy was performed immediately afterwards. Findings are summarized in Table II.

Dog IX. Summary of Protocol

When the experiment was begun, on May 19, 1941, dog IX, a male mongrel, weighed 9 kg. It was fed on a stock diet. Preliminary blood studies showed: opsonocytophagic index, 60; agglutination for brucella 456, negative; blood culture, negative. The animal was killed 186 days after the first injection.

Dog IX received 3 intraperitoneal inoculations of brucella (ABF 36 strain), each injection consisting of 10 billion organisms. The inoculations were given at intervals of 10 days. The last injection was given 152 days before death.

Opsonocytophagic index varied from 58.5 to 75. Agglutination of brucella 456, initially negative, rose to 1:2560 after 1 week and varied between this dilution and 1:10240. Brucella was recovered from the blood stream once in 5 cultures. The cultures were made from 2 to 11 weeks following inoculation. The positive culture occurred 3 weeks following the second injection.

During the experiment the dog remained well, and at no time were the peripheral lymph nodes enlarged.

Dog was killed with intracardiac ether (5 cc.); autopsy was performed immediately afterwards. Findings are summarized in Table II.

Dog X. Summary of Protocol

When the experiment was begun, on May 19, 1941, dog X, a female chow, weighed 8 kg. It was fed on a stock diet. Preliminary blood studies showed: opsonocytophagic index, 13.5; agglutination for brucella 456, negative; blood culture, negative. The animal was killed 216 days after the first injection.

Dog X received 3 intravenous injections of brucella (ABF 36 strain), each injection consisting of 10 billion organisms. The injections were given at intervals of 10 days. The last injection was given 182 days before death.

Opsonocytophagic index rose from 13.5 to 42 at the end of 1 week and varied between that figure and 71. Agglutination of brucella 456, initially negative, rose to 1:10240 after 1 week, and then remained at 1:20480. Brucella was recovered from the blood stream 3 times in 5 cultures made from 2 to 11 weeks following inoculation. The first positive culture was obtained 2 weeks following the first inoculation; other positive cultures were obtained 3 and 7 weeks following the last inoculation.

During the experiment the dog remained well, and at no time were the peripheral lymph nodes enlarged.

Dog was killed with intracardiac air; autopsy was performed immediately afterwards. Findings are summarized in Table II.

Experimental Results

A. Clinical Observations

From the protocols of the respective animals it is clear that a serious infection by brucella occurred in all four of the dogs inoculated intravenously with the Brody strain of organism. The infection was fatal in two of the four and doubtless would have been in a third had the intravenous inoculation been continued following the development of the profound intoxication that occurred in all of the animals in this group. The fourth intravenously inoculated animal in this group died early in the experiment from an acute intoxication produced by the ingestion of a mass of pine shavings that were being used for bedding. All of the animals of the group suffered from anorexia, pronounced loss of weight, great weakness and lassitude, and, finally, in the fatal cases, coma and death. Although the infection established in these dogs provoked the extraordinary clinical signs mentioned, it is evident from the experience with dogs II and IV that the dog has a remarkable resistance to infection by brucella and is capable of a quick and successful recovery from the acute manifestations of the disease when the inoculations are discontinued.

In spite of this great resistance to the infection, however, the organisms tend to persist in the tissues of the animal, particularly in the reticulo-endothelial system, where, as will be described shortly, they continue to provoke a morphological reaction. This is illustrated strikingly in dog II. Although this animal received the extraordinary number of 35 intravenous inoculations, the animal lived for 487 days and at autopsy was found to harbor brucella in the kidney and the lymph nodes. (No organisms had been received by this animal during the last 225 days of its life.) That the dog which has received a series of inoculations intravenously and has developed definite clinical disease is not protected against further inoculations after a rest period of considerable time is shown clearly in the experience with animal IV. This dog died following the resumption of inoculations with signs and symptoms similar to those which it had previously experienced. The disease of which these animals suffered and from which some of them died was a profound bacterial intoxication, as will be confirmed by the pathological anatomical findings to be recorded presently. This intoxication was not always accompanied by bacteremia. In fact, the

bacterial observations on these dogs showed that the dog is able to clear the blood of the inoculated organism within a relatively short time, usually within 3 weeks following the inoculation. After this time the organisms were found only in the tissues.

Judging from our experience with intraperitoneal inoculation of the dog, this route of infection is ineffective in establishing the clinical disease regardless of the number of inoculations administered. Nevertheless, blood cultures following intraperitoneal inoculations were positive on a number of occasions in two of the dogs so inoculated. In these animals the agglutination titers and the opsonocytophagic indices rose significantly, indicating a definite response of the tissues to the organism even though there were no clinical manifestations of disease. Our experience with intraperitoneal inoculation was the same for both strains of organisms employed.

It is worthy of comment that, whereas all the dogs inoculated intravenously with the Brody strain of brucella (an organism recovered from a case of Hodgkin's disease and of relatively low virulence for guinea-pigs) developed profound clinical disease, the one dog inoculated intravenously with the ABF 36 strain of brucella (an organism recovered from a spontaneous infection in a hog and of high virulence for guinea-pigs) developed no clinical evidence of infection. In spite of the absence of clinical disease in this animal, however, the organism was recovered from the lymph nodes at autopsy 182 days after the last inoculation was given. Active, subclinical infection in this animal is definitely indicated by the sustained elevation of the agglutination titer and the opsonocytophagic index, as well as by the demonstration of a positive blood culture 81 days following the last inoculation. The somewhat unexpected reaction of this dog possibly may be explained on the basis of the relatively few inoculations that were given; this animal received only 3 intravenous inoculations. This would seem to indicate that a relatively large number of inoculations, even though made intravenously, is necessary to establish clinical disease in the dog.

All of these clinical observations confirm the impression of previous workers that the dog is highly resistant to infection by brucella. At the same time, however, they indicate clearly that brucella in sufficient quantity is capable of producing even fatal disease in this animal, an observation which does not appear to have been made by previous workers.

B. Anatomical Observations

In the accompanying Table II are summarized all of the gross and histological findings in this group of animals. Of these findings only a few appear to be the result of the action of brucella. Outstanding

TABLE II

Experimental Brucellosis in Dogs: Autopsy Findings

Dog	Heart	Lung	Liver	Spleen	Lymph nodes	Kidney	Gastro-intestinal tract	Clinical disease	Days infected	Organisms: route and no. of inoculations
I	Endocardial hemorrhage, dilatation	o	o	o	R.E.* hyperplasia pronounced; hemorrhage, iron pigment; plasma cell reaction marked; focal epithelioid reaction	Epithelioid focal granulomata; chronic diffuse glomerulonephritis	o	Severe	198 died	Brody I.V. 21
II	Focal hemorrhage, dilatation	Foreign body; focal bronchial granulomata	Epithelioid granulomata; portal hepatitis	Focal hemorrhage; R. E. hyperplasia; many giant cells of bone marrow type; reticular scarring	Hemorrhage; R.E. hyperplasia	Focal scarring; dilatation of pelvis	o	Severe for 37 wks., clinical recovery after inoculations discontinued	487 killed	Brody I.V. 35
III	Focal hyaline degeneration	o	o	o	R. E. hyperplasia	o	o	o	461 killed	Brody I. P. 39
IV	o	Bronchial pneumonia; parasitic ova	o	o	Hemorrhage; R. E. hyperplasia, moderate	Acute diffuse hemorrhagic glomerulonephritis	Severe gastritis and enteritis	Severe	261 died	Brody I. V. 28

TABLE II (*Continued*)

Dog	Heart	Lung	Liver	Spleen	Lymph nodes	Kidney	Gastro-intestinal tract	Clinical disease	Days infected	Organisms: route and no. of inoculations
V	o	Confluent lobular pneumonia	o	Epithelioid focal granulomata	R. E. hyperplasia; granulomatous reaction	o	o	None; died from eating bedding	38 died	Brody I. V. 4
VI	Focal hyaline degeneration	o	o	Chronic capsular inflammation with scarring	R. E. hyperplasia, moderate	Focal granulomata	o	o	454 killed	Brody I. P. 39
VII	o	o	o	Capsular scarring; reticular scarring	R. E. hyperplasia pronounced	o	o	o	398 killed	Brody I. P. 33
IX	o	o	o	Reticular scarring; pulp sparse; hyperplasia of malpighian cells	R. E. hyperplasia pronounced; masses of mononuclear cells packing sinuses and replacing lymphatic cords	o	o	o	186 killed	ABF 36 I. P .3
X	o	o	o	o	R. E. hyperplasia with focal epithelioid reaction; iron pigmentation	Focal granulomata	o	o	216 killed	ABF 36 I. V. 3

* R.E. = Reticulo-endothelial.

among the various lesions are the following: (a) dilatation of the heart, (b) focal granulomata in the lymph nodes, liver, and kidney, (c) chronic inflammation and fibrous thickening of the splenic capsule, (d) hemorrhages or hemorrhagic pigmentation of the lymph nodes, (e) acute diffuse glomerulonephritis, (f) pronounced reticulo-endothelial hyperplasia in the lymph nodes, (g) intracellular brucella in the cells forming the sinusoidal reaction of the lymph nodes. Certain of these lesions are discussed briefly.

The most important of these lesions, as they relate to the primary objective of our experiment, is the rather remarkable sinusoidal reaction within the lymph nodes (Figs. 1 to 5). This lesion was found quite uniformly throughout the group of animals even though there was no gross enlargement of the lymphoid tissues. This sinusoidal reaction consisted of the proliferation of a mass of large mononuclear cells accompanied by an accumulation of a great number of polymorphonuclear leukocytes (Fig. 2). The lesion can be described best as a profound reticulo-endothelial reaction in which, as a rule, only the sinusoidal cells participate. However, in some of the nodes so affected a comparable reaction on the part of the reticulum of the lymph nodes occurred. The latter reaction resulted in the development of little foci of large, pale cells of distinctly epithelioid type (Figs. 3 and 4). Although these lesions were the exceptional finding, their presence seemed to leave no doubt that brucella is capable of exciting the growth of both the reticular and the sinusoidal elements of the reticulo-endothelial system. This reticulo-endothelial reaction, although prominent in virtually all of the nodes studied from all of the animals inoculated, is not quantitatively comparable to the reaction of the reticulo-endothelium that takes place in the lymph nodes of the guinea-pig when that animal is inoculated with brucella (Fig. 6); nor is the reaction comparable to that in the hog which we have described in a previous communication.[2] It is important to note that in the cytoplasm of these proliferating sinusoidal cells and the accompanying polymorphonuclear leukocytes, brucella in considerable quantity was demonstrated. This observation was made on the sections from an animal which had been inoculated 8 days before death (Fig. 2). In some of the lymph nodes showing the reticulo-endothelial reaction an extraordinary accumulation of plasma cells was found. These appeared in foci, usually situated outside of the sinuses and in the lymph cords. In lymph nodes showing this reaction the lesion as a whole resembled a genuine granulomatous reaction in its earliest phase of development (Fig. 5). Accompanying the reaction, multinucleated giant cells occasionally were found. These usually were not of the Langhans type. In some instances they resembled

somewhat the multinucleated giant cells seen in Hodgkin's disease. In all of the lymph nodes showing the sinusoidal reaction, variable quantities of fresh blood and iron pigment were found. Many of the sinusoidal cells contained phagocytosed materials of this sort. Accompanying these macrophages, there was always a great accumulation of the large reticulo-endothelial cells showing no evidence of phagocytosis. Although the changes described were pronounced in the lymph nodes in general, in none of these structures was there complete disturbance in the normal architectural relationships.

The little granulomatous foci in the kidney (Fig. 9), the liver (Fig. 10), and the spleen (Figs. 7 and 8) were found in only one or two of the animals. This lesion was especially prominent in the kidneys of one of the animals. Microscopically, the granulomatous focus was a collection of large mononuclear, sometimes epithelioid, cells accompanied by a few polymorphonuclear leukocytes and a few lymphoid cells. In some instances necrosis had occurred in the center of the lesion. No organisms could be demonstrated in any of these lesions. In general, the histological appearance of these little granulomata was identical with that of the foci of reticulo-endothelial hyperplasia found in the lymph cords of the lymph nodes. In view of the similarity of these lesions to those that one finds in brucellosis of the guinea-pig, and in view of the recovery of brucella by culture from nodes showing these lesions, it seems permissible to attribute the renal lesions to the brucella infection even though organisms could not be demonstrated in histological sections.

At this point it is necessary to call attention to the occurrence of focal granulomata of another type in the lungs of one of the dogs. These were quite clearly unrelated to the brucella infection; they were situated about the bronchi and always were associated with foreign bodies.

In two of the dogs the reticular structure of the spleen was particularly dense. This was unassociated with an increase in the number of pulp cells. The interpretation of this condition as a form of reticular scarring seems justifiable, but its relation to the brucella infection is difficult to establish.

The fibrous thickening of the splenic capsule occurred only in the animals inoculated intraperitoneally. Within the capsule at its thickest portion was found a mild chronic inflammatory reaction suggesting that the lesion was originally an inflammatory one (Fig. 8). Thus, it would appear that this lesion represents a brucella effect. No other alterations of the peritoneum that could be related to the inoculations were found.

All of the lesions other than those just discussed we have regarded as incidental findings or as the effects of the general intoxication produced by the long-continued brucella infection. One of these lesions, the diffuse glomerular injury to the kidney, is worthy of brief comment. This occurred in two of the animals and was unrelated to the development of the granulomatous foci. The histological changes found are like those customarily associated with acute and chronic diffuse glomerulonephritis in man. The route of inoculation in both of the animals showing this lesion was the same, that is, intravenous. These lesions are of particular interest in view of the fact that the experimental production of a typical acute diffuse glomerulonephritis by whatever method employed is a difficult accomplishment. The conditions of our experiment were such that one would have expected the development of a focal type of glomerulonephritis, a common form of injury to the kidney in all forms of bacteremia. It should be emphasized that the renal injury found in these two animals is unlike the usual spontaneous nephritis in the dog. All things being considered, it seems justifiable to attribute the nephritis in these animals to the experimental procedures. A discussion of the mechanisms involved in the production of injury to the kidney of the sort seen in these animals is not pertinent to our primary objective in this paper, and so we have dealt with the problem elsewhere.[5]

Comment

In these experiments it seems clear that we have succeeded in producing in the dog an infection by brucella that is recognizable both clinically and anatomically and which, in some instances, has been of sufficient severity to result in the death of the animal. That this has been accomplished only through the utilization of repeated inoculations of the organisms and that the anatomical alterations accompanying the infection are nondestructive and of an extremely mild character seem to indicate clearly that the dog is a highly resistant animal with tissues that are slow to react to infection by brucella. This observation is in harmony with the conclusions of previous workers. In planning our experiment, it had been hoped that the utilization of an animal that is so refractive to infection by brucella might make possible the production of long-standing chronic anatomical alterations in the tissues of the reticulo-endothelial system resembling the changes occurring in Hodgkin's disease. It is obvious that this has not been accomplished. At the same time, it is clear that a chronic infection by brucella does give rise to a pronounced alteration in the character of the lymphoid tissues of the dog. This alteration is the expression of a basic reaction on the part of the reticulo-endothelial cells that *in principle* may

be considered comparable to what occurs in Hodgkin's disease. If brucella is related etiologically to Hodgkin's disease, a possibility which certain recent observations seem to suggest, there must be certain peculiar and highly important factors involved in the relationship that the experimental studies of ourselves and others have not yet disclosed. Theoretically, it appears entirely possible that such factors may exist.

Summary and Conclusions

1. By means of repeated intravenous inoculations of a strain of *Brucella suis* obtained from a case of Hodgkin's disease, a severe, sometimes fatal, form of chronic brucellosis has been produced in dogs. Dogs so affected have been observed for as long as 487 days.

2. It has not been possible to produce clinical disease in dogs by repeated intraperitoneal inoculations of a strain of *Br. suis* obtained from a case of Hodgkin's disease. This was true also when the inoculations were made with a strain of *Br. suis* obtained from a naturally infected hog. Both of these strains of brucella were known to be pathogenic for guinea-pigs.

3. Clinical brucellosis in the dog is characterized by anorexia, loss of weight, weakness, lassitude, and coma. The course of the disease is progressive only so long as the inoculations are continued. The dog is highly resistant to the infection and may recover from the most severe infection if the inoculations are discontinued.

4. Dogs repeatedly inoculated either intravenously or intraperitoneally and without clinical disease may harbor virulent brucella in the tissues of the reticulo-endothelial system for as long as 7 months after the inoculations are discontinued.

5. The most constant anatomical alterations resulting from brucella infection in the dog are found in the lymph nodes. These consist of a pronounced reticulo-endothelial reaction involving both the sinus endothelium and the reticulum cells of the lymphatic cords, without significant enlargement of the nodes. The result of this reaction is the development of focal granulomata of epithelioid character in the lymphatic cords and the formation of great masses of large mononuclear wandering cells which fill and eventually replace the lymphatic channels. Similar focal granulomata of epithelioid character occur occasionally in the kidney, liver, and spleen. A variety of nonspecific lesions occur, including petechial hemorrhages, focal hyaline degeneration of the heart muscle, and acute gastro-enteritis. These appear to be the result of bacterial intoxication.

6. Repeated intravenous inoculations of *Br. suis* produced anatomically typical, acute, diffuse glomerulonephritis in two of four dogs.

7. Prolonged brucella infection in dogs gives rise to a marked pro-

liferative reaction on the part of the reticulo-endothelial system of a granulomatous character, but it does not produce an anatomical alteration of these tissues comparable to that which characterizes human Hodgkin's disease.

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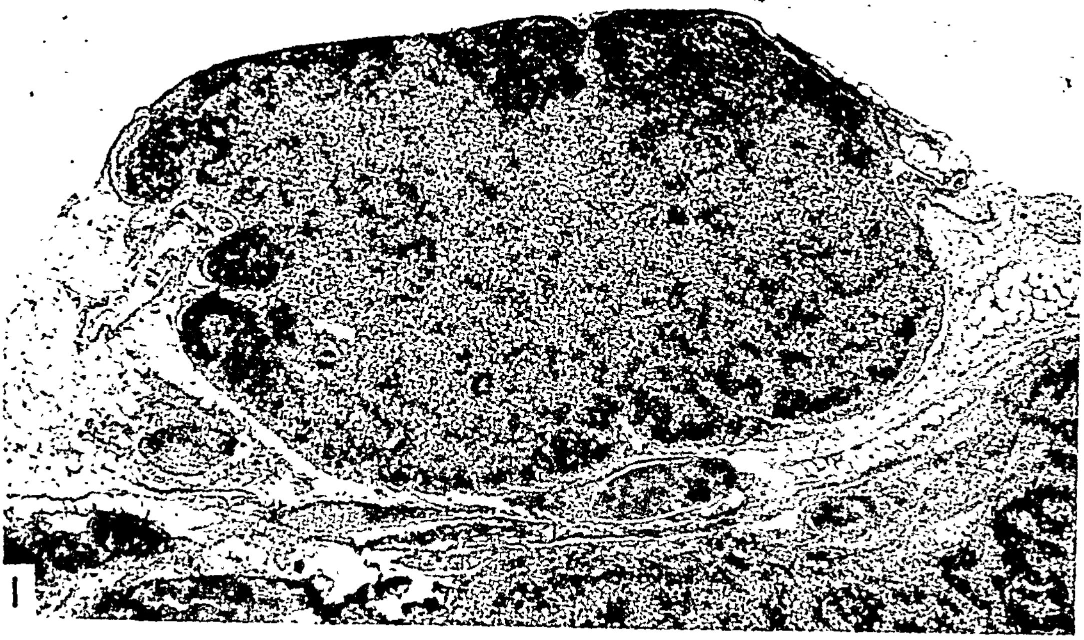
DESCRIPTION OF PLATES

Plate 127

Fig. 1. Lymph node from dog V. This animal had received 4 intravenous inoculations of *Brucella suis* (Brody strain) which had been obtained originally from a case of Hodgkin's disease of 5 years' duration. This low-power photomicrograph shows a widespread sinusoidal reaction and replacement of the lymphoid cells by new cells. This reaction is typical of all the inoculated animals. Figures 2, 3, and 4 show the cell types involved in the reaction. $\times$ 5.

Fig. 2. Early sinusoidal reaction consisting of the accumulation of polymorphonuclear leukocytes and reticulo-endothelial hyperplasia. The lymph node is the same as shown in Figure 1. Only a few of the proliferating reticulo-endothelial cells are active phagocytes. Brucella was demonstrated in the macrophages and the polymorphonuclear leukocytes. $\times$ 485.



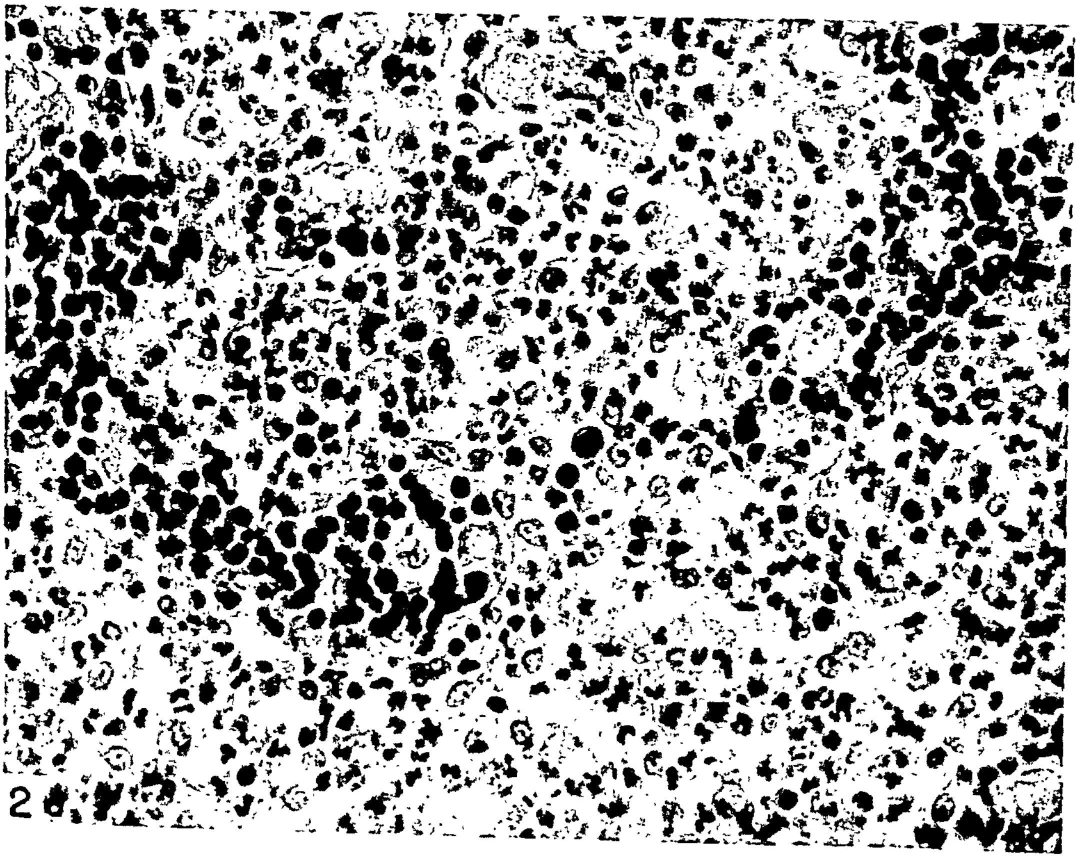


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PLATE 128

FIG. 3. Epithelioid transformation of the proliferating reticulo-endothelial cells in a lymph node from dog IX. This animal had received 3 intraperitoneal inoculations of *Br. suis* (ABF 36 strain), originally recovered from a naturally infected hog. The reaction is a genuine granuloma and is identical with that which occurs in the guinea-pig (Fig. 6.) A lower power view of the node from which this photograph was made is shown in Figure 5. × 365.

FIG. 4. Focal proliferation of the reticulum cells of a lymphatic cord accompanied by a marked sinusoidal reaction in a lymph node from dog I. For comparison with the guinea-pig reaction shown in Figure 6.

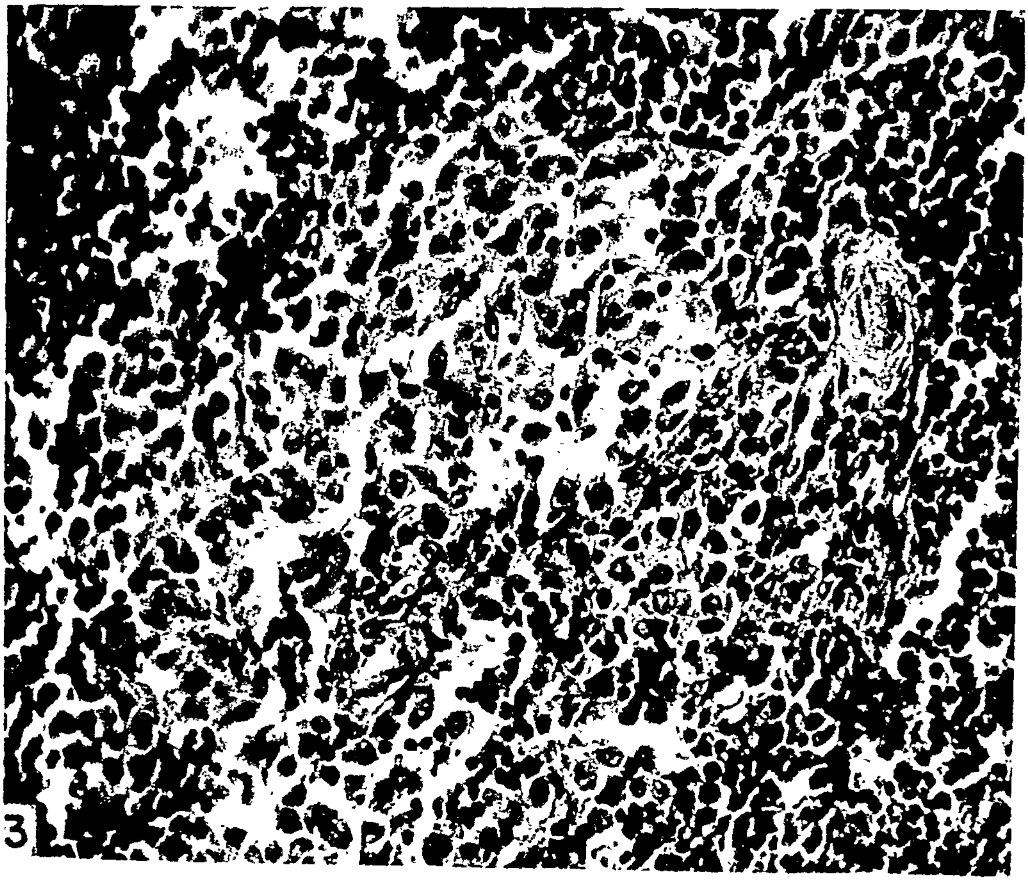

PLATE 129

FIG. 5. Typical early reaction of the lymph nodes accompanying infection by *Br. suis* (ABF strain). This lymph node from dog IX was virtually replaced by the reticulo-endothelial reaction; grossly it resembled the node shown in Figure 1. Although the cells vary greatly in morphology, the reaction at this stage is not epithelioid except in scattered foci. These foci are pictured in Figure 3. In some areas the foci coalesce and produce a picture not unlike that in the guinea-pig reaction (Fig. 6). × 157.

FIG. 6. A typical granulomatous transformation of the lymph node in a guinea-pig infected with *Br. suis* for comparison with the reaction in the dog's node. × 137.

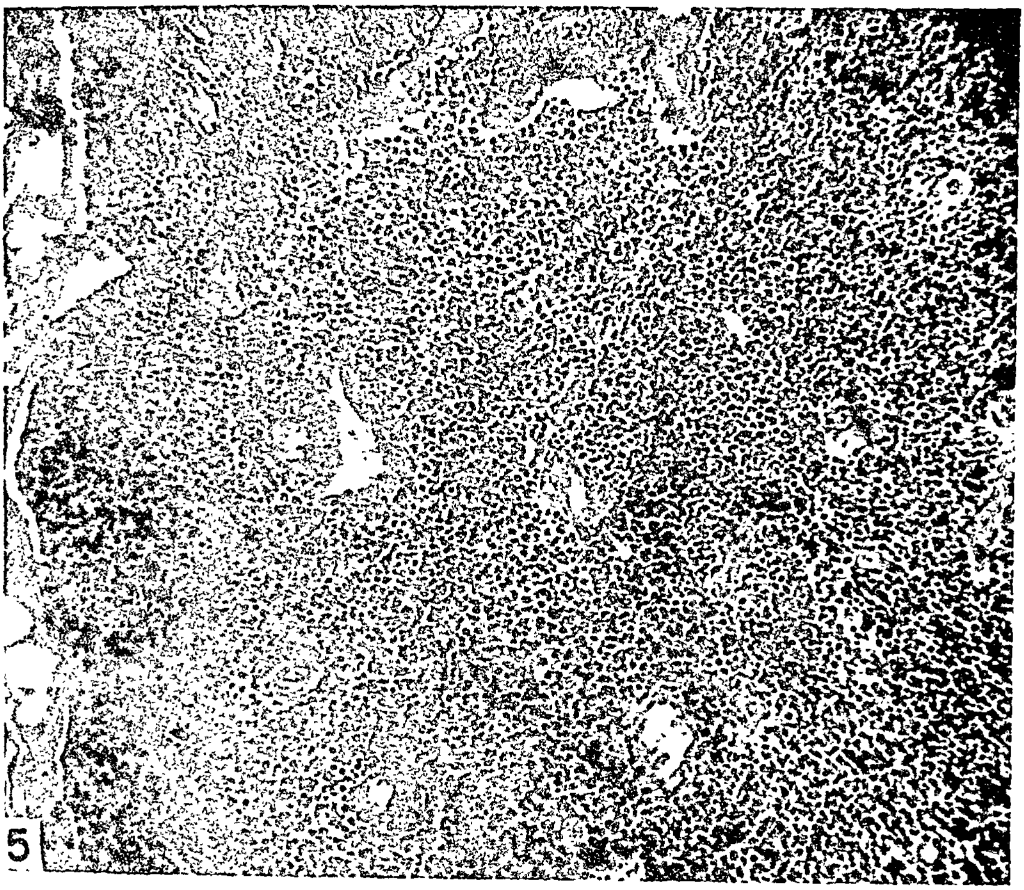

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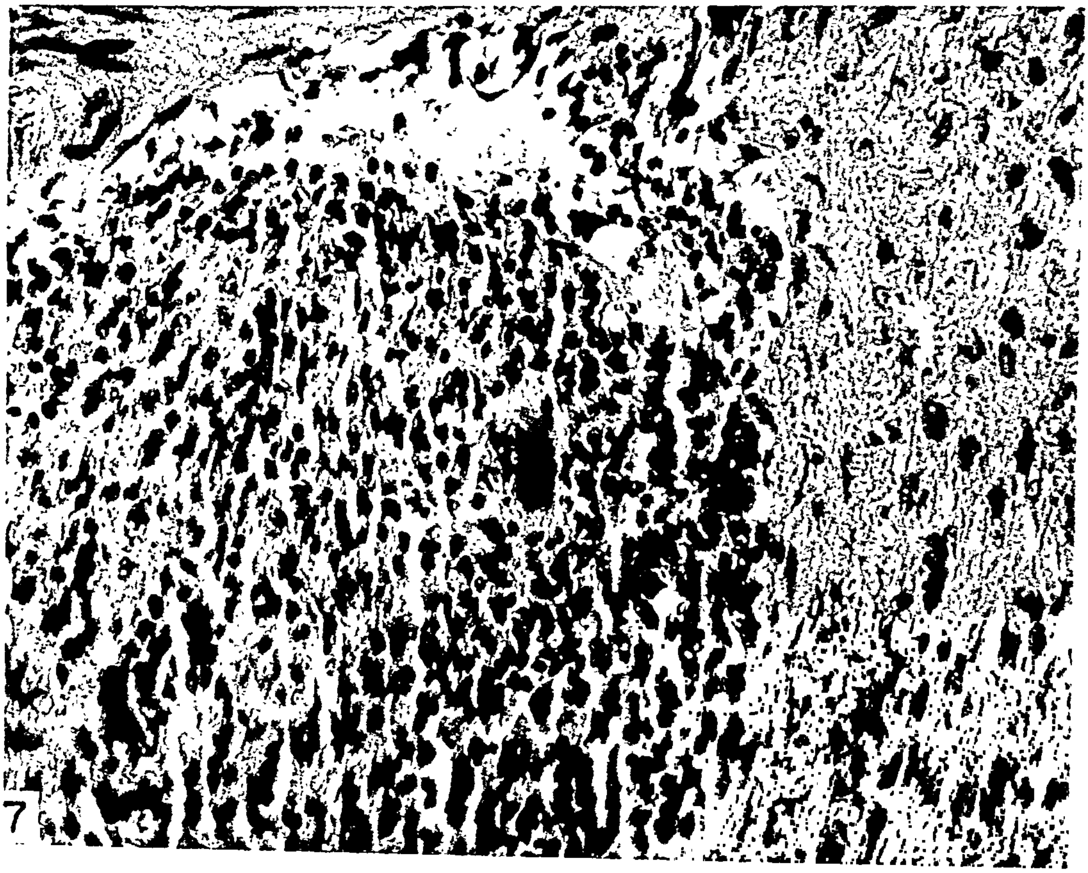
PLATE 130

FIG. 7. A small granulomatous focus in the spleen just beneath the capsule, with a large giant cell of megakaryocytic type. Giant cells of other types, some with one nucleus and others with several nuclei such as those pictured in Figures 2 and 4, are more common in the typical reaction to brucella. Dog VI, from which this section was taken, received 39 intraperitoneal inoculations of *Br. suis* (Brody strain) and lived for 454 days. The lymph nodes showed the reaction pictured in Figure 1, and the kidneys showed focal granulomata like that pictured in Figure 9. × 375.

FIG. 8. A necrotic inflammatory lesion in the capsule of the spleen of dog VI. The section is from the spleen shown in Figure 7. × 182.



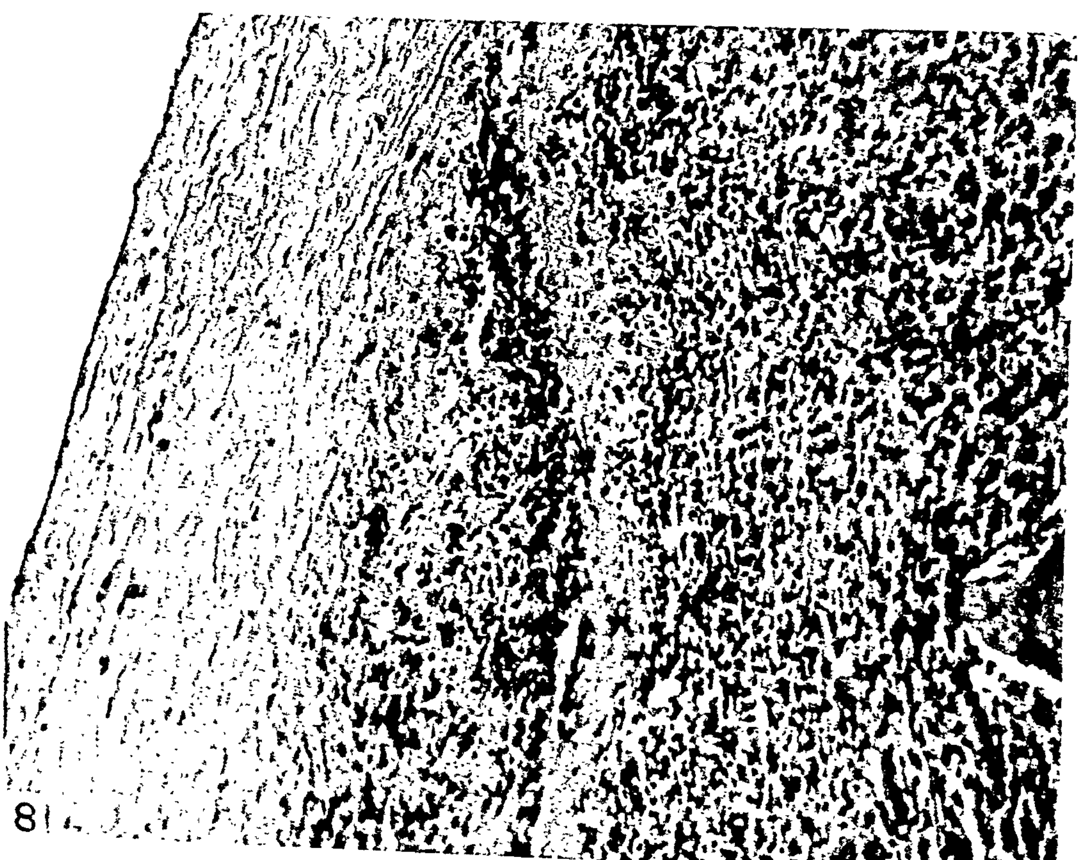


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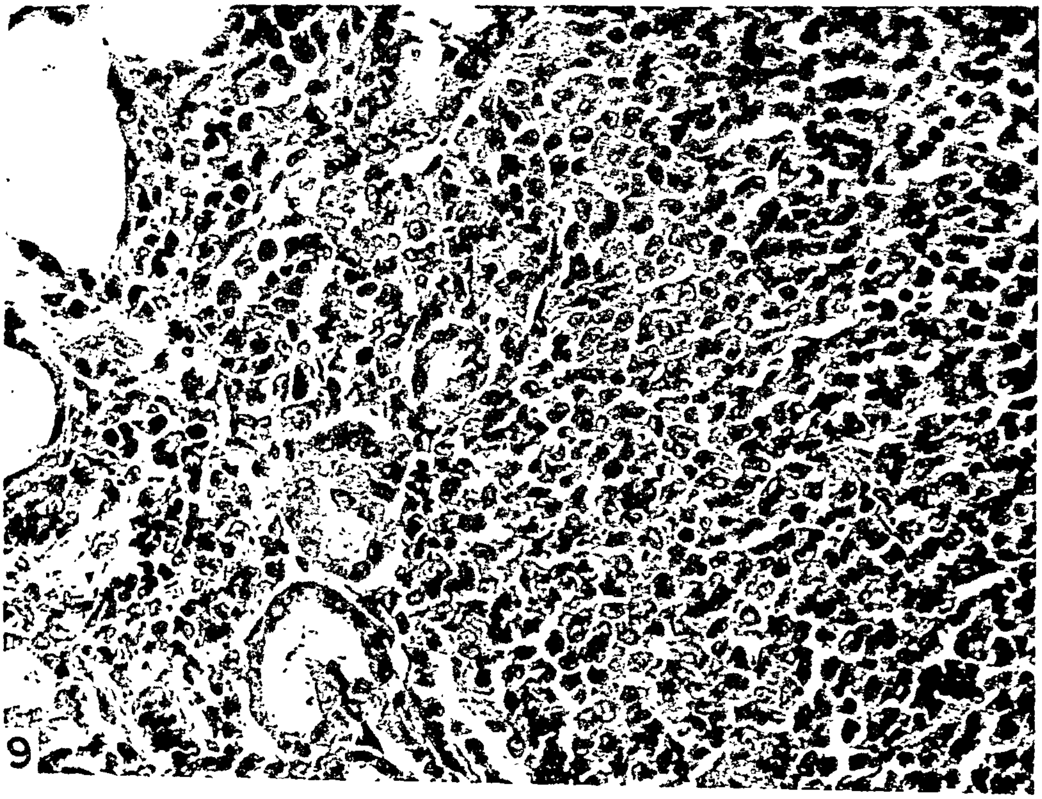
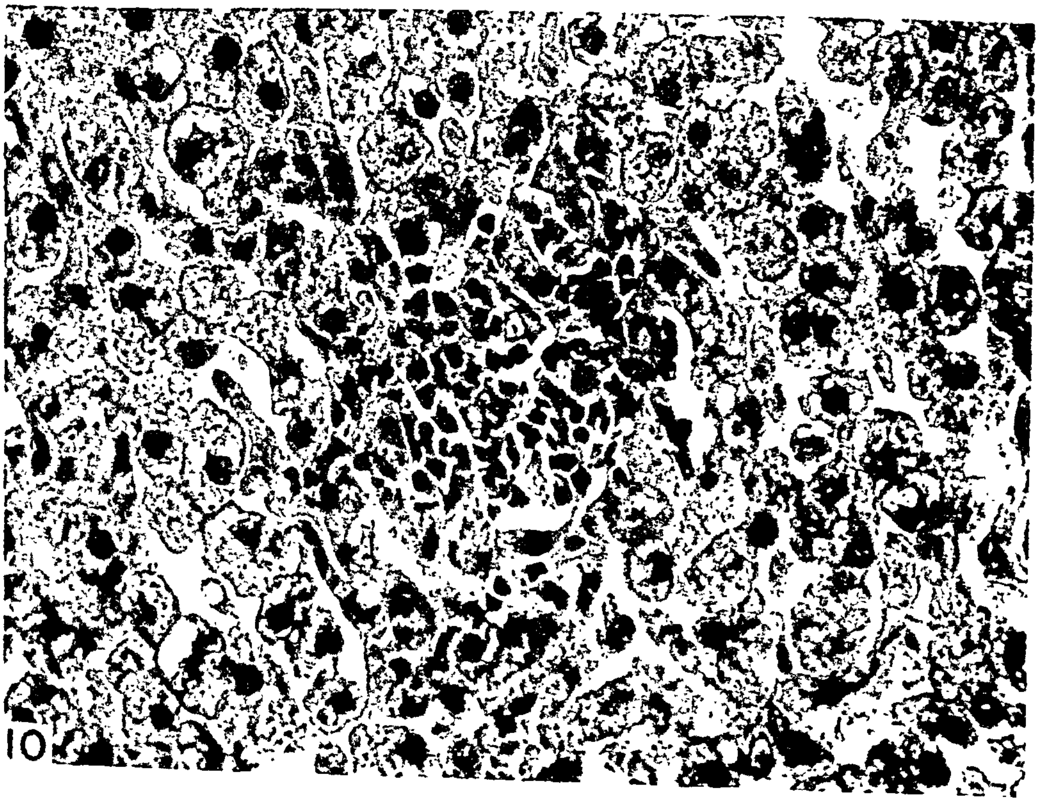
Plate 131

Fig. 9. Focal granuloma in the kidney of dog I. The typical reacting cells are reticuloendothelial, but there is also a scattering of polymorphonuclear leukocytes; many of these are eosinophils. Lesions of this type were numerous in two of the dogs. × 365.

Fig. 10. Focal granuloma in the liver of dog II. This animal had received 35 intravenous inoculations of *Br. suis* (Brody strain) and was killed 487 days after being infected. Lesions of this sort are like those seen in the guinea-pig liver. They are not numerous. × 485.





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Experimental Brucellosis of Dogs

FAILURE OF PRESSOR DRUGS TO INFLUENCE "JUXTAGLOMERULAR APPARATUS" IN RATS *

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The observation of special afibrillar or granular cells in the "juxtaglomerular apparatus" of the normal kidney of various species [1-7] has led to speculation concerning the rôle of these cells. At first these cell groups were regarded as modified smooth muscle cells, somewhat analogous to myo-epithelioid cells of the myo-arterial glomus. Ruyter,[1] who first described these cells, suggested that they may modify the blood flow through the arteriole and glomerulus by imbibition and swelling, with consequent narrowing of the arteriolar lumen. Some observers [6, 7] have accepted this view. Later Elaut [8] reported hyperplasia of this cell group in dogs rendered hypertensive by denervation of the carotid sinus and cardio-aortic zones. Goormaghtigh and several coworkers found an increase in number and size of "these large, afibrillar and sometimes granulated or vacuolated cells" in renal hypertension produced by Goldblatt clamps in dogs and rabbits [9] and in experimental hypertension due to hypervitaminosis D (calciferol).[10] Goormaghtigh [11] postulated a "glandular cycle culminating in the formation of acidophil or basophil secretion granules intermingled with minute vacuoles" and concluded "that the endocrine activity of the afibrillar cells is related to the production of hypertensive substance present in the ischemic kidney." Dunihue and Candon [12] confirmed the observations in rabbits with renal hypertension [12, 13] and accepted Goormaghtigh's explanation. Hypertrophy of these cells was found by Kaufmann[14, 15] in human kidneys from hypertensive subjects. He did not find an increase in the number of cells and specified that they were large clear cells that contained a few granules, neither more definite nor distinct than they were in "normal" kidneys. Oberling,[16] in a recent report on human kidneys, objected to Elaut's and Goormaghtigh's observations on kidneys from hypertensive patients and claimed that the latter misinterpreted mastocytes and histiocytes for granular cells. Instead, Oberling reported that the preglomerular granular cells are always "degenerated or completely destroyed" in the malignant forms of hypertension.

In the absence of physiologic data supporting any of these hy-

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potheses, we undertook to test the function of the granular cells by administering pressor drugs known to act on the arterioles. Morphologic changes were sought in the character, number, and distribution of these cells as well as changes in other visceral and peripheral arterioles.

The rat was chosen because it is relatively easy to make repeated determinations of blood pressure by indirect methods and because the "juxtaglomerular apparatus" is well defined. Large granular cells are easy to demonstrate in one-fifth to three-fifths of the afferent arterioles as they enter the glomeruli in "normal" adult rats.

The blood pressure was measured at intervals before and throughout the period of drug administration to determine if chronic hypertension could be induced. As pressor substances most likely to affect the granular cells, angiotonin, tyramine hydrochloride, epinephrine hydrochloride, and pituitrin were chosen.

Angiotonin * (Page,[17] or hypertensin [18]) may be of etiologic importance in hypertension and has been shown to have special vasoconstrictor properties. Tyramine has long been suspected to be a pressor amine formed in the ischemic kidney [19-21] and its necrotizing effect on the vessels has been recorded.[22] Experiments extending from 1 to 282 days were performed with these two preparations.

Experiments were conducted for from 1 to 30 days using the more active pressor drugs. Epinephrine was used because of its constrictor effect on the renal afferent arterioles,[23-25] pituitrin because of its ability to produce vascular lesions in the kidney, stomach, and other organs.[26, 27] Another reason for examining the renal vessels was the report of augmented responses to adrenalin, tyramine, and pitressin in rabbits with renal ischemia.[28]

Methods

Altogether 50 normal rats were used, weighing from 150 to 370 gm. Males and females were about equal in number in each group. A mixed diet was fed (Purina checkers). At least three control determinations were made of the blood pressure of each animal by the indirect method of Byrom and Wilson [29] under anesthesia (sodium pentobarbital, 0.5 mg. per kg. intraperitoneally). To avoid the depressor effect of the anesthetic the animals were gently heated for 3 minutes in an environmental temperature of 40°C. during anesthesia.[30] The systolic pressure was determined twice weekly during the experimental period.

Numerous blood pressure determinations were made prior to and

* We are indebted to Dr. Irvine H. Page of the Lilly Clinic, Indianapolis City Hospital, who kindly supplied the angiotonin.

after the administration of the drug and during the pressor effect. The animals were sacrificed by inhalation of ether usually 1 hour after the last injection. Specimens of organs were fixed in Zenker's solution and serial sections through the greatest diameter of the kidney were stained with Goldner's modification * of Masson's trichrome stain and Mallory's phosphotungstic acid hematoxylin. The latter stain proved very helpful in detecting the granular cells. From different histologic sections of the kidneys of each animal twenty clearly visible afferent arterioles were studied as they entered the glomeruli. The presence of granular or clear cells was noted and any deviation in thickness or structure of the media was recorded.

Seventeen animals were given *angiotonin* in doses from 0.03 to 0.2 cc. intraperitoneally once or twice a day except Sundays for periods of 1 to 189 days. From 2 to 125 injections were administered. Interruptions of 2 to 8 days were sometimes necessary when prompt shipment of the drug was impossible.

Nineteen rats were injected with *tyramine hydrochloride* in doses of 8.75 to 25.0 mg. per kg. intraperitoneally. Injections were given once or twice daily except Sundays for periods of 2 to 282 days, to a total of 5 to 413 injections.

A 1:1000 solution of *epinephrine hydrochloride* was given to 6 animals in doses of 0.25 mg. per kg. intraperitoneally once or twice a day; 1, 5, 10, 20, and 40 injections respectively were given in periods of 1 to 30 days. In this group an attempt was made also to detect changes in the granular cells caused by the final injection of the drug. Therefore one kidney was removed before the last injection was given and the other one immediately after the blood pressure had reached its peak.

Eight animals were given 3, 9, 18, and 30 injections of *pituitrin S* (Parke, Davis & Co.) subcutaneously three times daily for 1 to 10 days. Each injection contained 5 international units.

Control experiments were done with physiologic saline solution injected intraperitoneally.

Results

The doses of the pressor drugs produced restlessness, erection of the hair, pallor of the skin of the ears and toes, an increase of respiratory rate, and, after tyramine hydrochloride, augmented salivation and often a marked exophthalmos. A steep rise of 20 to 125 mm. of Hg in the systolic blood pressure followed every injection of *angiotonin, tyramine hydrochloride, epinephrine hydrochloride,* and *pituitrin S* and reached

* Goldner, J. A modification of the Masson trichrome technique for routine laboratory purposes. *Am. J. Path.*, 1938, 14, 237–243.

a peak within 1 to 3 minutes. The decline of the pressure was equally rapid.* Control experiments with physiologic saline solution injected intraperitoneally were without any pressor effect. The pressor response to *angiotonin* was 20 to 70 mm. of Hg; to *tyramine hydrochloride*, 40 to 113 mm. of Hg; and after *epinephrine hydrochloride*, 50 to 125 mm. of Hg. Increases which followed *pituitrin* injections ranged from 10 to 125 mm. of Hg. In none of the animals did the systolic pressure fail to return to levels recorded prior to the administration of the drug.

In neither the short nor the prolonged experiments in the four groups was there any significant change in the granular cells of the renal afferent arterioles. These cells, if present, were limited to 2 or 3 in the media at the termination of the afferent arteriole (juxtaglomerular apparatus or Polkissen †). They were counted in 20 terminal arterioles of the kidneys of each group. The 17 animals which received *angiotonin* showed granular cells present in 2 to 13 (average 8) instances; they varied from 3 to 12 (average 6) in the 19 animals of the *tyramine* group; and from 6 to 15 (average 10) in the kidneys removed just before the last injection of *epinephrine* was given. The granular cells were found in 7 to 13 (average 8) Polkissen of 20 arterioles in the kidneys extirpated after the last injection of epinephrine.

The 8 animals treated with *pituitrin S* had the lowest range, with 3 to 7 granular cells per 20 terminal arterioles; the average was 5. There was no relation between the number of granular cells and the number of injections in any of the four groups.

In 7 animals given *tyramine* the granular cells appeared swollen and were found in the proximal part of the afferent arteriole. This finding bore no relation to the number of injections, which ranged from 5 to 373 in these instances. A similar observation was made in 1 animal of the *adrenalin* series after 10 injections.

There were other histologic findings that could not be attributed to the effect of any of the drugs. More or less marked vacuolization of the medial cells in the interlobular arteries of the kidneys was very frequent and the degree could not be related to the number of injections.

In 2 animals of the *tyramine* group there were microscopic areas of

* There is a possibility that the apparent steep decline recorded by the plethysmographic method is due to progressive constriction of the tail arteries, thus yielding apparently lower readings. Intra-arterial measurements are necessary to verify this point.

† This refers to the collection of cells in the wall and sheath of the afferent arteriole visible where it enters the glomerulus. Adjacent to it is the macula densa, a palisade arrangement of the cells in the distal convoluted tubule attached to the same nephron.

focal necrosis in the myocardium, the spleen, and liver (in the latter two probably due to *Salmonella enteritidis* infection). Another animal of this group had dry gangrene at the tips of the tail, penis, and toes after 190 injections. However, no distinctive vascular lesions were present in the histologic sections of these areas.

The subcutaneous injections of *pituitrin S* caused necrotizing myositis and marked arteritis at the site of the injection. In only 1 rat, after 30 injections of pituitrin S, were there other arterial lesions found in the viscera; in this instance there was proliferation of medial and adventitial cells in some pancreatic arterioles.

Comment

The negative results presented here neither support nor deny Goormaghtigh's hypothesis of a glandular cycle in the "juxtaglomerular apparatus" with a pressor rôle in renal hypertension. They merely show that these cells are not modified by the action of pressor drugs. They also show that tyramine hydrochloride and angiotonin, given over periods as long as 282 and 189 days respectively, do not produce a permanent elevation of the systolic pressure. It is possible that by giving the same dosage over longer periods or by using larger dosage in the same period, both renal vascular changes and hypertension might be induced. The evidence in the relevant literature is inconclusive in regard to the blood pressure. Enger and Lampas [31] injected dogs intramuscularly with tyramine in amounts as large as 3.0 gm. daily for a period up to 2½ years and found increases in the basal systolic pressure of only 10 to 20 mm. of Hg; their histologic findings were negligible in all organs examined. Duff, Hamilton, and Magner [22] injected 50 to 100 mg. of tyramine into rabbits over a period of 1 to 106 days, and obtained vascular lesions in the brain, kidney, heart, larger arteries, and arterioles. They did not report measurements of the blood pressure of these animals.

Enger [32] injected 0.2 to 50 mg. of epinephrine into dogs daily up to 2 years. He found narrowing of the retinal arteries with thickening of their walls, and hyalinization of the capillaries of the glomerular tuft. Penner and Bernheim [33] in short experiments on dogs, cats, guinea-pigs, and rabbits used much larger doses than we did; they gave 0.3 to 4.2 mg. per kg. of epinephrine intraperitoneally and produced ulcerative lesions in the digestive tract of rabbits and guinea-pigs. These authors succeeded in producing bilateral cortical necrosis of the kidney when they injected 0.3 to 0.5 mg. per kg. of epinephrine into dogs for from 6 to 30 days.[34]

Dodds, Noble, and Smith [27] produced severe necrosis of the fundic

region of the stomach of rabbits by injecting 200 to 800 units of pituitrin in a single dose or a few massive doses. Using the rat they recommended the oral route of administration as the most successful and pointed out that "very large doses" are necessary to obtain gastric ulceration by subcutaneous injections (personal communication). Byrom,[26] however, injected 5 to 40 units of pituitrin subcutaneously into rats daily for several weeks and observed blanching attributed to arterial spasms of the living kidney, and infarction of kidneys and other organs, as well as necrosis in liver and arteries. Although these doses were approximately the same as those given by us, we were unable to find similar changes except for those seen at the site of injection. Nedzel [35] injected 20 units of pituitrin per 5 kg. into dogs twice weekly up to 50 injections and obtained gastric and duodenal ulcers as the result of vascular injury in 23 of 60 dogs. With the exception of the results obtained by Byrom,[26] the changes were produced either with doses considerably larger than ours or in species other than the rat.

Summary

In short (1 day) and prolonged experiments (up to 282 days), repeated doses of angiotonin, tyramine hydrochloride, epinephrine hydrochloride, and pituitrin were given to 50 rats in an attempt to influence the granular cells of the renal afferent arterioles in the so-called "juxtaglomerular apparatus."

A steep rise of the systolic pressure followed every injection but no sustained elevation above its basic level could be induced. No significant changes in the number, localization, or appearance of the granular cells or the medial cells of the renal arterioles could be found.

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PRIMARY INTRACRANIAL CHORIONEPITHELIOMA WITH METASTASES TO THE LUNGS *

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Chorionepitheliomas, which usually arise in the uterus or ovaries and less frequently in the testes, may, exceptionally, have their origin outside the reproductive organs. Most of the relatively few authentic cases of extragenital chorionepithelioma probably originated from teratomas which underwent unilateral differentiation. Intracranial teratomas, arising most frequently in the region of the pineal body, have been reported occasionally. We find, however, only two reports [1, 2] of primary intracranial chorionepithelioma.† Both cases are subject to the criticism that other possible primary sites were not adequately investigated.

The first case, which was reported by Askanazy,[1] was that of a boy, 19 years old, who had at autopsy a hemorrhagic tumor the size of a walnut in the region of the corpora quadrigemina. The tumor secondarily involved the vermis and dentate nucleus of the cerebellum. The pineal body was not identified and no metastases were noted. Histologic examination of the tumor removed from the brain disclosed a typical chorionepithelioma with cells of both syncytial and Langhans' types. The thoracic and abdominal viscera contained no tumor, but unfortunately the testicles were not sectioned. Askanazy justified his diagnosis of chorionepithelioma of the pineal body on the facts that (1) no large masses or nodules were observed in the scrotum by the clinician or pathologist, (2) pineal metastases are rare, and (3) serial sections of the pineal tumor showed no evidence of vascular dissemination. The possibility that there might have been a small primary tumor in the testicles which gave rise to a large metastasis in the brain, as mentioned by Askanazy, is not conclusively disproved. It is a well established fact that patients with small primary chorionepitheliomas may have large metastases.

The second case of chorionepithelioma reported as primary within the cranial cavity was that of Wirth.[2] The patient was a boy, 6 years

* Received for publication. August 26, 1944.

† After this manuscript was submitted, Davidoff published a brief report of a patient with a chorionepithelioma of the pineal body (Davidoff, L. M. The endocrinologic aspects of tumors of the pineal gland. *Surgery*, 1944, 16, 306–314). The patient, a boy, 9 years old, with signs of macrogenitosomia praecox, had a tumor in the region of the pineal body on which a diagnosis of chorionepithelioma was made from microscopical sections of the tumor, supported by a positive Friedman test. Unfortunately, post-mortem examination was limited to the head so that the possibility of primary or metastatic tumor in other organs cannot be excluded.

old, with precocious puberty. The autopsy study revealed a hemorrhagic mass with linear gray areas involving the splenium of the corpus callosum, the corpora quadrigemina, the anterior medullary vellum, and the lingula of the cerebellum. The tumor measured 28 by 48 mm. The histologic description was that of an atypical chorionepithelioma, but no microphotographs were published. There were no metastases. The left testicle was normal, but the right testicle and the thoracic and abdominal viscera were not examined. The fact that the tumor was microscopically atypical and the limited extent of the post-mortem examination leave the diagnosis of primary chorionepithelioma of the pineal body subject to considerable doubt.

In the case reported here, the diagnosis of intracranial chorionepithelioma was made ante-mortem and thorough post-mortem examination and hormone analyses were performed to establish the primary source and nature of the tumor. This case is of especial interest because it is one of the few proved instances of primary extragenital chorionepithelioma and the first with origin in the region of the pineal body definitely established.

Report of Case

The patient, a white boy, 15 years old, was admitted to the Barnes Hospital (no. 99,552) on August 8, 1942. His chief complaints were vomiting for 2 weeks, dizziness, double vision and frontal headache for 10 days, and convulsions for 3 days. He had been well until 2 weeks before, when he became listless and began vomiting. At this time he developed a staggering gait and, following lumbar puncture, it was noted that his speech was slurred. He was in a semicomatose condition when admitted to the hospital. The significant physical findings noted at that time were slight papilledema of the left eye, weakness of the left side of the face, bilateral paralysis of the sixth nerve, and diminished activity of the deep tendon reflexes. The family history, past history, and laboratory examinations showed nothing unusual. Roentgenologic examination of the skull before and after injection of air into the ventricles disclosed a calcified tumor in the region of the pineal body and a defect in the posterior part of the third ventricle.

On August 11th, a transcallosal exploration was undertaken. Because of the small size of the ventricles the exposure was very difficult. A small amount of tissue thought to be tumor was removed. Histologic examination of this tissue revealed only normal choroid plexus. Because the patient's condition failed to improve, on August 17th a Torkildsen operation[3] was performed in which a catheter was inserted between the lateral and fourth ventricles in an attempt to circumvent the block in the third ventricle. Following this operation some of the symptoms were slightly improved for a few days, but then the patient's condition became progressively worse.

On September 8, 1942, left craniotomy was performed and a solid hemorrhagic-appearing tumor was removed from the region of the pineal body. Following the operation, the patient remained stuporous and developed a huge cerebral hernia, making it necessary to tap the ventricles every few days. He then developed typical decerebrate rigidity and was fed by nasal tube. The temperature and pulse remained elevated but the respirations were regular. On October 14th, the patient

developed edema of the left leg and signs of bilateral pleural effusion, and harsh râles were heard at the bases of the lungs. Histologic examination of the tumor removed at the last operation disclosed a typical chorionepithelioma. The urine was examined and found to contain large quantities of gonadotropic hormone. The patient died on October 18th, 6 weeks after his last operation.

AUTOPSY REPORT

Autopsy (Department of Pathology, Washington University, no. 10,085) was performed 2 hours after death. The body was well developed but somewhat emaciated and there was slight edema of the left lower extremity. The nipples were more prominent than usual but there was no increase in mammary tissue. The growth and distribution of hair and the size and form of the external genitals were normal. In the scalp over the left parietal region were the healed linear wounds of the craniotomies. Beneath the scalp in this region was a fluctuant mass. On reflecting the scalp, it was found that the fluctuant mass was a part of the left cerebral hemisphere with the overlying meninges herniated through the operative defect in the calvarium. Brownish yellow fluid escaped from the herniation. Some of the fluid was collected for the determination of the presence of hormones. A small rubber catheter extended from one lateral ventricle through the calvarium and beneath the scalp to the region of the foramen magnum where it entered the fourth ventricle. A thrombus was noted in the superior sagittal sinus, but the other dural sinuses were normal.

The weight of the brain was moderately increased, being 1670 gm. The left parietal and temporal lobes of the brain were enlarged, the cerebral convolutions were flattened, and the corresponding sulci were partially obliterated. A sagittal section through the brain disclosed a mass of reddish brown neoplastic tissue completely filling the third ventricle and compressing the hypothalamus inferiorly and splenium of the corpus callosum superiorly. The tumor measured 4.5 by 4.5 by 7.0 cm., and was sharply outlined from the surrounding brain tissue (Fig. 1). As a result of its downward and posterior extension the cerebral peduncles were compressed and nearly completely transected. The surface of the tumor was finely granular and mottled red and white. The pineal body was not identified. The lateral ventricles were moderately dilated. The tentorium and falx cerebri in the region of the incisura were infiltrated with reddish brown neoplastic tissue of the same type as was noted in the brain. The spinal cord was normal and no tumor was identified during the removal of the spinous processes or around the cord.

The cut surfaces of all lobes of the lungs showed soft, white and

firm, red nodules which protruded slightly above the surrounding parenchyma. The largest nodules measured 2 cm. in diameter. Pink, friable thrombi were found in the secondary and tertiary arterial branches in all lobes. Extending to the pleural surface of the lower lobe of the right lung there was a firm, red, hemorrhagic area measuring 3 cm. in diameter. The artery leading to this area contained a red thrombus. The lower lobe of the right lung was firm, noncrepitant, and darker red than the surrounding lung.

The retroperitoneal tissues and lymph nodes were examined carefully at autopsy but no tumor was identified. After fixation the testes and epididymides were cut into slices and carefully examined without finding any discolorations, scar formation, or evidences of tumor. All abdominal and thoracic viscera were removed, fixed in formalin, and cut into 1 to 2 cm. slices in a meat slicing machine. Blocks were taken for microscopic examination from all areas suggestive of neoplasm.

Microscopic Examination

Sections of the intracranial tumor removed at autopsy presented the typical appearance of chorionepithelioma as shown in Figures 2 and 3. The tumor was composed of cells of two distinctly different types. The predominating type was a large cell with single or multiple nuclei and coarse strands and clumps of chromatin. In certain areas masses of the large cells were surrounded by the smaller second type of cell arranged in a single thin syncytial layer. This arrangement of the large and small cells resembled a chorionic villus (Fig. 2). Many extensive areas of hemorrhage and necrosis were noted throughout the tumor. The invasiveness of the tumor was shown by its penetration of adjacent brain tissue in a few areas and of the falx cerebri and superior sagittal sinus. Although sections were examined from more than 45 serial blocks of the intracranial tumor, no other evidence of a teratoma was found, and cells of the pineal body were not identified. In several of the sections there were, however, many spherical calcified bodies identical with those usually seen in the pineal body. It was concluded that the calcified bodies were of pineal origin and that all normal pineal tissue had been destroyed by the tumor.

A section through one of the hemorrhagic-appearing nodules in the lungs showed hemorrhagic, necrotic neoplastic tissue of the same type as was seen in the brain. There was invasion of the subpleural lymphatics by tumor cells and the overlying pleura was thickened and covered by a thin deposit of fibrin. The tumor was actively invading the surrounding alveoli which were partially collapsed and contained erythrocytes and a few large macrophages (Fig. 4).

A section of breast tissue showed a definite increase in the number of lining cells with an increased thickness of the ductal epithelium. A few ducts contained plugs of desquamated epithelial cells.

There was no secondary spermatogenesis in the seminiferous tubules of the testis. Between the tubules were many interstitial cells with abundant eosinophilic cytoplasm. Although microscopic sections were prepared from 47 blocks, selected from serial areas throughout the testes not more than 2 mm. apart, neither a tumor nor a scar of a healed tumor was found. There was no squamous metaplasia of the epithelium of the colliculus seminalis or utricle of the prostate gland. The pituitary gland showed a slight increase in the number of acidophilic cells.

Pathologic Diagnoses. The diagnoses were: chorionepithelioma, primary in the region of the pineal body and involving the floor of the third ventricle, with extension to the tentorium, falx cerebri, and the superior sagittal sinus; metastatic chorionepithelioma in all lobes of the lungs; hyperplasia of the mammary ducts, slight; herniation of the left cerebral hemisphere through the operative defect in the calvarium; partially organized thrombi in the pulmonary arteries, superior sagittal sinus, inferior vena cava, and left iliac and periprostatic veins; edema of the left leg, slight; recent infarct of the lower lobe of the left lung; bronchopneumonia of the lower lobes of the lungs; hydrothorax, bilateral (200 cc. on the right and 700 cc. on the left); superficial ulceration of the skin over the sacrum; lipoidosis of the aorta, slight; fatty infiltration of the myocardium; fibrous nodules in the liver; calcified nodules in a right tracheobronchial lymph node; multiloculated cystic cavities in the spleen with calcified contents.

HORMONE ANALYSES

On the day of the patient's death, specimens of urine and of cerebrospinal fluid from the lumbar region and from the lateral ventricle were collected. Four cc. of each of these solutions was injected intravenously into a virgin female rabbit. After 24 hours, several corpora hemorrhagica were present in each ovary. This indicated the presence of abnormal amounts of gonadotropins in the urine and cerebrospinal fluid.

At autopsy, 185 cc. of urine, 56 cc. of the cerebrospinal fluid, and 2.3 gm. of the metastatic tumor from the lungs were saved for analysis. The gonadotropins and estrogens were each separated from the urine according to the technic of Levin and Tyndale,[4] from the cerebrospinal fluid by the method of Delfs,[5] and from the neoplastic tissue by the procedure of Parker and Tenney.[6]

Gonadotropin determinations were made by Dr. Willard Allen by observing the response in the ovaries of mature rabbits after the injection of measured amounts of the extracts. With the methods employed a rabbit unit was found to be equivalent to approximately 5 international units of chorionic gonadotropin. The urine contained the equivalent of 310 to 435 rabbit units per liter while the cerebrospinal fluid had between 25,590 and 38,400 rabbit units per liter. The extract of gonadotropins from the neoplastic tissue of the lung had an activity of between 3,480 and 4,350 rabbit units per kg. of tissue.

The extract of the estrogenic fraction of the equivalent of 48 cc. of urine dissolved in sesame oil and injected into one ovariectomized mature rat produced proestrus changes in the vaginal epithelium. Previous observations had shown that 1.25 gammas of estrone was necessary to produce estrus in 50 per cent of the rats. The injection of the equivalent of 14 cc. of cerebrospinal fluid produced no change in the vaginal epithelial cells.

As a more sensitive test for estrogens, injections of the extracts in sesame oil were made into immature female rats about 20 days old. Using a standard technic three injections were made within 24 hours and the rats were killed 20 hours after the last injection. The change in uterine weight was determined by comparison with litter mate controls. In a similar manner, the increase in uterine weight produced by known amounts of estrone was determined. The mean body weights of comparable groups of rats varied only a few grams. Usually 3 or 4 rats were used in each group of injected and control animals. Because of the small amount of material available for analysis from the metastatic tumor, only 1 rat was injected with the total extract. These assays showed that 1,000 gm. of tumor contained the equivalent of 370 gammas of estrone. Similarly, 1,000 cc. of urine and of cerebrospinal fluids would have an activity of 90 and 30 gammas of estrone respectively.

Discussion

Of especial interest relative to this case are the hormonal secretions, the incidence and origin of extragenital chorionepithelioma in men and women, and the metastases of intracranial tumors.

Hormones

The results of the analyses of the hormones in this case are similar to the findings published by other workers. Because the fluids for analysis were inadvertently kept overnight at room temperature before the extractions were started, the results obtained may be somewhat lower than might have been found under more favorable conditions.

The amount of gonadotropins present in the urine was similar to the lower levels found in pregnant women.

Zondek [7] cited a luteinizing action of more than 416 mouse units per liter of spinal fluid as an important diagnostic point for chorionepithelioma. Each liter of this patient's cerebrospinal fluid contained between 130,000 and 190,000 international units of chorionic gonadotropic hormone. This unusually large amount of gonadotropins obtained is partially accounted for by the fact that the cerebrospinal fluid was in direct contact with the neoplastic tissue of the brain.

Zondek [7] considered 100 mouse units of luteinizing substance per gm. of extra-uterine tissue as sufficient to establish the diagnosis of metastasizing or extragenital chorionepithelioma. In this case there was the equivalent of between 17,000 and 22,000 international units of chorionic gonadotropins per kg. of pulmonary metastatic tumor. The hormones may be decreased in necrotic neoplastic tissue [7] such as was used in these analyses.

Some workers [7] have doubted that patients with chorionepithelioma have increased amounts of estrogenic substances as a result of the tumor. Other investigators,[8, 9] however, have claimed that the estrogens are significantly increased with chorionepithelioma although the amount of estrogenic substances produced is generally much less than is found in pregnant women. Twombly and Hocker [10] found more estrogens in the urine of a man with a chorionepithelioma of the testis than in the urine of any other normal male or menopausal female patient whom they tested. Gilbert [11] also reported abnormal amounts of estrogen in 7 cases of chorionepithelioma.

The urine of an adult male contains an average equivalent of 7 gammas of estrone per liter.[12] Our results show an increase of thirteen times this amount of estrogenic substances in the urine. The cerebrospinal fluid, and especially the metastatic neoplastic tissue, also contained abnormal amounts of estrogens.

Gynecomastia has been reported in 10 per cent of the cases of chorionepithelioma of testicular origin.[13] Although the estrogens may produce hyperplasia of the mammary ducts, as was observed in our patient, in this instance the amount of estrogenic substances present was either small or was present for only a short period of time, because there was no squamous metaplasia of the epithelium of the colliculus seminalis or prostatic utricle such as occurs with increased amounts of estrogenic substances. Smith and Smith [8] showed that the chorionic cells themselves, when they become neoplastic, do not contain amounts of estrogen comparable to those found in normal placentas. Whether the estrogenic hormones came from the chorionepithelioma or from the

adrenals, which were morphologically normal, is not known. The demonstration of pregnandiol in the urine suggested to Twombly and Hocker [10] that the adrenals of their patient had been stimulated abnormally and this stimulation perhaps was the explanation of the increased estrogens.

Although changes in the pituitary gland resembling those in pregnancy have been reported in many cases of chorionepithelioma, only a slight increase in the number of acidophils was observed in our case. The significance of the increased number of interstitial cells in the testicle, which has been described in chorionepithelioma and was present to a moderate degree in this case, is not understood. The absence of secondary spermatogenesis can be explained by the patient's debilitated condition and does not necessarily represent a result of excessive estrogens. Perhaps if the primary tumor had been situated where it interfered less with the patient's vital functions, he might have survived longer and have shown more advanced changes evidencing hormonal imbalance.

Extragenital Chorionepithelioma

The literature contains 26 reports of men [2, 13] and 16 reports of women [14] in whom chorionepithelioma was thought to be of extragenital origin. In most of these cases the genital organs were inadequately examined. Prym,[15] in 1927, reported an instance of a chorionepithelioma of apparent extragenital origin in which there was a small scar in one testicle that in his opinion represented a healed primary testicular tumor. However, trauma of the testicle is a frequent cause of small scars in the testis, whereas the evidence of spontaneous healing of chorionepithelioma may be questioned. It is not clearly understood how the primary growth of such a malignant neoplasm, even though it is associated with hemorrhage and necrosis, could spontaneously regress while the metastases continued to grow.

Following this report by Prym,[15] authors have become more critical and have refused to accept multiple sectioning of the testicle as indisputable proof of the absence of primary testicular tumors unless multiple microscopic sections, not more than 2 mm. apart, are prepared from various areas throughout the testicle. The reports of chorionepithelioma in which the testicles are examined according to such rigid criteria fall into three groups: (1) those which contain no evidence of tumor; (2) those which contain teratoid vestiges; [16] and (3) those with easily demonstrable testicular tumor.

Erdmann, Brown, and Shaw [13] reported 1 case and accepted the 4 published cases of Fenster,[17] Gerber,[18] Kantrowitz,[19] and Weinberg [20] as conclusively proved instances of chorionepithelioma in men, not

primary in the testicle. In all of these cases the testicles were examined by the method of serial block sections and there was absolutely no evidence of a primary tumor or scars to suggest that the primary tumor had healed. It is entirely possible that there are additional instances of extragenital chorionepithelioma among the cases reviewed by Erdmann, Brown, and Shaw in which there were insufficient data to establish the case beyond criticism.

Of the 26 possible cases of extragenital chorionepithelioma in men reported and reviewed in the literature,[2, 13] 17 were believed to be primary in the abdomen, 7 in the thorax (of which 6 were in the mediastinum), and 2 in the region of the pineal body. Prym [21] cited the observation of Greiling that all primary testicular tumors metastasize to the retroperitoneal lymph nodes as evidence against the presence of ectopic chorionepitheliomas primary in retroperitoneal tissues. However, the retroperitoneal tissues are also the frequent site for the development of teratomas and of embryonic rests derived from the urogenital fold. Of the 26 extragenital chorionepitheliomas reported in men, it was claimed that 13 were primary in the retroperitoneal tissues and 1 each in the aortic lymph nodes, lung, and liver. Thus, about one-half of the reports of extragenital chorionepitheliomas do not mention a tumor in the lymph nodes or retroperitoneal tissues as one would expect if they were all metastatic from testicular tumors.

In 1940, Berman [14] reported a case of extragenital chorionepithelioma in a woman and reviewed 15 other cases with autopsies from the literature. In almost all instances the tumor was associated with pregnancy. The fact that in 14 of these 16 cases tumor was present in the lungs suggests that it is the usual "primary" or intermediate site for the development of extragenital chorionepithelioma in women. These observations have been used to support the concept that extragenital chorionepithelioma results from the malignant transformation of chorionic tissue which has been transported to the lungs.[14]

Although extragenital chorionepithelioma could arise from teratomas or embryonic rests in women as well as in men, it is more difficult to establish indisputably such an origin in women. The distribution of the neoplastic tissue in the reported ectopic chorionepitheliomas in women is essentially the same as the location of metastases in established cases of primary genital chorionepithelioma; that is, most frequently in the lungs, liver, and brain.[22] Unlike the extragenital chorionepitheliomas in men, in no instance was neoplastic tissue found in the retroperitoneal tissues or mediastinum, which are common sites for teratomas and the two most frequently claimed sites of primary extragenital chorionepithelioma in men.

Novak and Koff [23] described a case of a woman, 31 years old, in whom examination of curettings showed chorionepithelioma. The patient subsequently died of metastatic chorionepithelioma of the brain. There was no tumor in the uterus at autopsy and one must assume that a primary uterine tumor, after metastasizing to the brain, was either removed by the curettage or regressed spontaneously. That chorionepithelioma may at times undergo spontaneous healing in women is believed by some authors.[17, 24]

Wilson [24] reported a case (no. 3) of a woman, 28 years of age, who developed neurologic signs and symptoms and died. At autopsy there was chorionepithelioma in the brain, lungs, spleen, and kidneys but no evidence of this tumor in the uterus or ovaries. In such cases it is impossible to ascertain whether there had been a primary uterine tumor which disappeared after metastases had developed or whether it developed as an ectopic tumor from trophoblastic cells transported during a previous and perhaps unrecognized pregnancy. For these reasons we will give no further consideration to extragenital chorionepithelioma in women.

Origin of Extragenital Chorionepithelioma

It is generally believed that the chorionepitheliomas primary in the testicles develop from previously existing teratomas, but in a large number of cases it is not possible to identify the teratoma. In those cases in which the teratoma is not identified it is thought that the neoplastic chorionic tissue has overgrown and completely destroyed the teratomatous elements. The concept that is generally held regarding these tumors is that they arise from the undifferentiated totipotential cells found in teratomas. Cases have been reported of chorionepitheliomas that apparently arose from teratomas in the ovary, mediastinum, and retroperitoneal tissues. Some authors [18] think that germinal rests along the urogenital anlagen may give rise to retroperitoneal chorionepitheliomas, and Staemmler [25] has reported the occurrence of testicular rests in the retroperitoneal fat at the root of the mesenteric vessels. Since the plica urogenitale extends from the sixth thoracic to the second sacral segments in the embryo, it is conceivable that germinal rests might be present in the mediastinum in the adult.

Sweet [26] reviewed 156 cases of dermoid, teratoid, and teratomatous intracranial tumors reported in the literature prior to 1940. He found a total of 44 teratomas, of which 28, or 63.6 per cent, were situated in the region of the pineal body, 4 were in the pituitary region, and 3 in the posterior part of the posterior cranial fossa. These are the three most frequent sites of intracranial teratomas. Occasionally, teratomas are

observed in the choroid plexus of the ventricles, but the more frequent sites of origin within the cranial cavity are in the region of the pineal body and of the pituitary gland.

Although the lungs and the liver are the most frequent sites for metastases from chorionepithelioma, the brain is also frequently involved. We know of only 2 cases [1, 2] in which it was claimed that a chorionepithelioma was primary within the cranium and in which no evidence of tumor was reported elsewhere in the body. As already mentioned, in these cases the examination for possible tumors of the testis was inadequate. In instances where tumor is present in other organs, as well as the brain, the burden of proof rests upon the author to show that the tumor within the brain is not metastatic.

Extracranial Metastasis of Intracranial Tumors

Metastases from primary intracranial tumors are extraordinarily rare. Several cases with extracranial metastases from intracranial fibroblastic tumors (arachnoidal fibroblastoma) have been reported.[27, 28] Instances of extracranial metastases from gliomas are less well established than from arachnoidal fibroblastomas (meningioma) although there are two reasonably well substantiated cases.[29] The case presented here is an instance of a primary intracranial tumor that produced extracranial metastases and adds interesting information to the general question of the observed infrequency of extracranial metastases from intracranial tumors. The chorionepithelioma is a tumor notorious for its ability to metastasize, and the metastases are usually blood-borne. Our case and the cases reported by Russell and Sachs [27] and Abbott and Love [28] demonstrate clearly that tumors that are known to have the ability to metastasize show this tendency when they are primary within the cranial cavity.

Discussion of Reported Case

We believe that our patient had a teratoma in the region of the pineal body from which developed a rapidly growing chorionepithelioma as a result of the differentiation of one component of the teratoma. The chorionepithelioma then destroyed the original teratoma as well as all normal tissue. The intracranial tumor invaded the superior sagittal sinus and other blood vessels and metastasized by way of the venous blood to the lungs.

In our opinion the possibility that the tumor was of testicular origin was eliminated by the careful examination of the testicles, as previously described. If a primary tumor of the testicle were present, it was less

than 1 mm. in diameter and lacked the characteristic red color of a chorionepithelioma or the firm white consistency of a scar of a healed tumor. A most careful search of gross sections of all of the other organs, which were cut into slices of 1 to 2 mm. in thickness, showed that no tumor more than 2 mm. in diameter was present except in the brain and lungs.

That the tumor nodules in the lungs were metastatic rather than primary is indicated by the absence of demonstrable tumor in the mediastinum or near the hilum of the lungs where teratoma and extragenital chorionepithelioma have been reported. Moreover, teratomas are rare tumors of the lungs and the peripheral location of the tumor nodules is characteristic of metastatic tumor. The demonstration of neoplastic cells within the veins about the tumor in the brain offers an obvious explanation of the manner of the production of the pulmonary metastases.

In favor of the pineal body as a site of the primary tumor, in addition to the negative evidence of any other primary source, is the fact that teratomas of a type known to give rise to chorionepithelioma are frequently observed in the region of the pineal body. The observation of a single focus of metastatic tumor of the brain located in the pineal body would be most unusual. For these reasons the case here reported is thought to be an authentic example of an extragenital chorionepithelioma, an established case of primary intracranial chorionepithelioma, and one of the few examples of primary intracranial tumor with extracranial metastases.

Summary and Conclusions

The case of a 15-year-old boy with a primary chorionepithelioma in the region of the diencephalon with metastases to the lungs is reported. The histologic structure of the tumor was typical of chorionepithelioma. Quantitative analyses of hormones showed significant amounts of gonadotropins and estrogens in the urine, cerebrospinal fluid, and metastatic neoplastic tissue. It is believed that the chorionepithelioma arose in the region of the pineal body from a previously existing teratoma. The testes and other organs were eliminated as sites for a possible primary growth of the tumor by thorough sectioning.

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DESCRIPTION OF PLATE

PLATE 132

FIG. 1. Medial sagittal section of the brain, showing a large hemorrhagic and necrotic tumor of the diencephalon.

FIG. 2. Section from the surgically removed portion of the tumor in the pineal region showing structures resembling chorionic villi composed of cells of syncytial and Langhans' types. Hematoxylin and eosin stain. × 430.

FIG. 3. Section from the tumor in the pineal region, obtained at autopsy, showing syncytial cells with large hyperchromic nuclei and the numerous mitotic figures in Langhans' cells. Hematoxylin and eosin stain. × 395.

FIG. 4. The hemorrhagic, metastatic chorionepithelioma is invading the parenchyma of the lung and the subpleural lymphatics. Hematoxylin and eosin stain. × 70.

1

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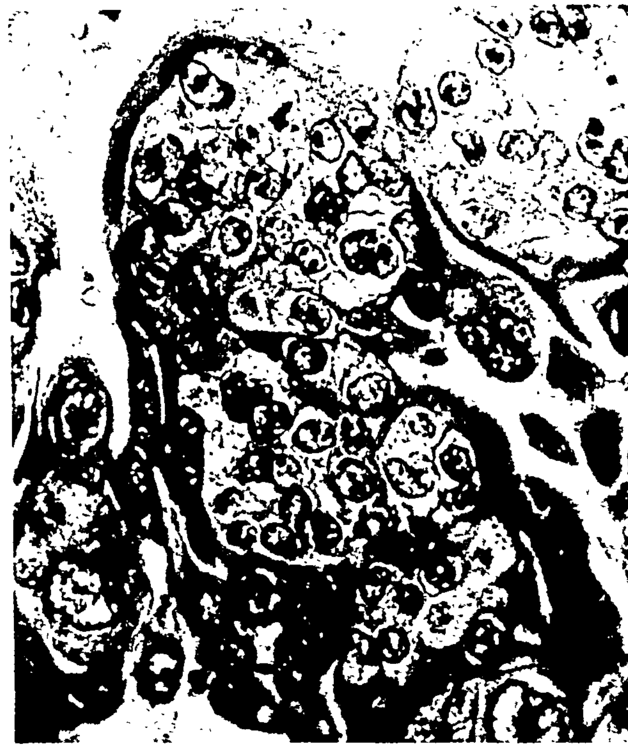

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4

Stowell, Sachs, and Russell

Primary Intracranial Chorionepithelioma

RENAL INJURY IN THE RAT FOLLOWING THE ADMINISTRATION OF SERINE BY STOMACH TUBE *

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The kidneys of animals have been injured experimentally by various means. Among the studies on this subject are a fairly large number utilizing various dietary factors as nephrotoxic agents. Weanling rats placed on diets low in choline have developed degenerative kidney disease, and Griffith and Wade [1-3] have shown that injury in this instance can be prevented by the addition of choline to the diet. Recently György and Goldblatt [4] found that the specific injurious effects of choline-deficient diets on the kidney were aggravated by the addition of pyridoxine. Hartwell [5] and also Cox and Hudson [6] have produced typical renal abnormalities in young rats by feeding them diets deficient in vitamins B. Newburgh and Curtis [7] have shown that renal lesions can be produced in animals by feeding them large amounts of certain kinds of proteins. Employing rabbits and dogs, Newburgh and Marsh [8] found that the intravenous administration of the amino acids, arginine, aspartic acid, lysine, histidine, tyrosine, tryptophane, and cystine, resulted in severe renal necrosis, while other amino acids gave no evidence of a necrotizing action on renal tissue. Later Lillie,[9] utilizing the material of Sullivan, Hess, and Sebrell,[10] found that lysine, tyrosine, tryptophane, cystine, and glutathione were nephrotoxic to young rats maintained on a diet containing 4 per cent casein as the main source of protein. The nephrotoxic action of free cystine in the diet of young rats had been previously demonstrated by Cox, Smythe, and Fishback.[11]

In the course of experiments on the dietary factors affecting the composition of phospholipids in tissues, Fishman and Artom [12] noted an injurious action of *dl*-serine administered by stomach tube in rats maintained on a synthetic diet deficient in protein and in the B vitamins. This injury was characterized by anorexia, sudden loss in weight, marked weakness and a high mortality. Similar animals on a stock diet showed only transitory and slight ill effects as a result of the administration of the amino acid.

The purpose of this paper is to report a series of anatomical studies in white rats to which the amino acid, serine, was administered by stomach tube. The report deals further with the influence of a diet deficient in B vitamins and in protein on the experimental lesions.

Recent attempts at intravenous alimentation with mixtures of amino

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acids and of protein hydrolysates add interest to the investigation of the possible toxic action of individual amino acids.[13] Further, the resemblance of some of the experimental renal lesions to certain types of degenerative kidney disease in man is of interest and may possibly have an etiological significance.[4]

Material and Methods

White male rats weighing 95 to 105 gm. were divided into four groups. The rats in group 1 (Table I) were maintained on a stock diet (Rockland Farms Rat Diet "Complete") * and received daily 100 mg. of *dl*-serine † in 3 cc. of water by stomach tube. The majority of the

Table I
Stock Diet Plus Serine

Rat	Total days on experiment	No. days receiving serine	Degenerative kidney lesions*	Liver, fatty infiltration
A-21-1	1	1	+	–
A-21-2	1	1	+	–
A-25-1	3	3	+	–
A-25-2	3	3	+	–
A-21-3	7	7	+	–
A-21-4	7	7	+	–
A-25-5	11	11	+	–
A-21-6	11	11	+	–
A-13-1	23	14	+	–
A-13-2	23	14	+	–
A-13-3	33	14	+	–
A-13-4	33	14	+	–

* See description in text.

animals were sacrificed at various intervals from 1 to 14 days after the first administration of the amino acid. Other animals which had received *dl*-serine for 14 days were allowed to continue on the stock diet alone and were killed 9 and 19 days respectively, after the last administration of the amino acid.

The second group of animals (Table II) was placed on an experimental diet (diet 4) [14] composed of "Labco" vitamin-free casein,‡ 10 parts; dextrin, 37; sucrose, 37; Crisco,§ 5; cod-liver oil, 5; "Ruffex," || 2; salt

* Rockland Farms Rat Diet "Complete" is a diet composed of ingredients of both animal and plant origin (cane molasses, soy bean meal, fish and meat scraps, various grain preparations, milk products, seed oils, yeast, etc.) The chemical composition is protein, 24.8 per cent; fat, 4.7 per cent; carbohydrate, 49.3 per cent; fiber, 4.8 per cent; ash, 9.5 per cent. Further details may be obtained from the Arcady Farms Milling Co., Chicago, Ill.

† Pure racemic *dl*-serine, $CH_2OH.CHNH_2.COOH$ was obtained from Merck and Co., Rahway, N.J.

‡ Labco vitamin-free casein is a highly purified casein which is guaranteed to be free of all vitamins and is made by the Borden Co., New York, N.Y.

§ Crisco is a partially hydrogenated vegetable oil made by Proctor and Gamble, Cincinnati, Ohio.

|| Ruffex is a cellulose material containing no fats, vitamins, or proteins and is made by the Fisher Scientific Co., Pittsburgh, Pa., especially for use with experimental diets.

mixture (Osborn and Mendel *), 4. In order to bring about as uniform experimental conditions as possible they received in addition 3 cc. of water daily by stomach tube for a maximum period of 14 days. The

TABLE II

Experimental Diet Alone

Rat	Total days on diet	Degenerative kidney lesions	Fatty infiltration of liver
A-24-1	8	Intracytoplasmic hyalinization of tubular epithelium	−
A-24-2	8	Intracytoplasmic hyalinization of tubular epithelium	−
A-24-3	13	Minimal chromatolysis of tubular epithelium	−
A-24-4	13	Minimal chromatolysis of tubular epithelium	−
A-24-5	20	Minimal chromatolysis of tubular epithelium	−
A-24-6	20	Minimal chromatolysis of tubular epithelium	−
A-23-4	20	Calcification, fibroblastic and mononuclear response	+
A-23-5	20	Calcification, fibroblastic and mononuclear response	+
A-4-1	30	Calcification	+
A-4-4	30	Calcification, diffuse cortical necrosis	+
A-5-2	40	Calcification, marked	+
A-5-3	40	Calcification, minimal	−
A-5-4	40	Normal kidneys	+

rats were sacrificed at varying intervals, after having been on the experimental diet for periods of time ranging from 8 to 40 days.

The third group of animals (Table III) was placed on the experi-

TABLE III

Experimental Diet Plus Serine

Rat	Total days on experiment*	No. days receiving serine	Degenerative kidney lesions	Fatty infiltration of liver
A-20-1	1	1	Necrosis, marked	−
A-20-2	1	1	Necrosis, marked	−
A-12-1	3	3	Necrosis, early calcification	+
A-12-2	3	3	Necrosis, early calcification	+
A-11-2	4	4	Necrosis, early calcification	−
A-11-3	4	4	Necrosis	+
A-12-6	4	4	Necrosis, calcification	+
A-12-3	6	6	Necrosis, calcification, repair	+
A-12-5	6	6	Necrosis, calcification, dilated tubules, repair	+
A-12-4	6	9	Necrosis, calcification, dilated tubules, repair	+
A-20-4	14	14	Calcification, repair nearly complete	−
A-11-1	23	14	Calcification, repair nearly complete	+
A-11-4	23	14	Calcification, repair nearly complete	−
A-11-5	33	14	Calcification, areas of scarring	+
A-11-6	33	14	Calcification, areas of scarring	+

* Not including the first 7 days on the experimental diet.

* Osborne, T. B., and Mendel, L. B. The relation of growth to the chemical constituents of the diet. *J. Biol. Chem.*, 1913, 15, 311–326.

mental diet described above and allowed to continue for a period of 7 days. The administration of 100 mg. of *dl*-serine by stomach tube was then started and the amino acid given daily for a maximum of 14 days. The animals were then sacrificed at varying intervals, and several rats were allowed to continue on the diet for 2, 9, and 19 days after the amino acid had been discontinued.

The fourth group of animals, consisting of 4 rats, was utilized as controls; they were maintained on the stock diet until they had reached a weight of 100 gm. or more and were then sacrificed.

In agreement with previous results,[12] several of the animals of group 3 died during the first week of serine administration. No deaths occurred in the rats of the other groups. The surviving animals were killed by decapitation, and autopsy was performed immediately. Multiple blocks were taken from all organs, and fixation was done in duplicate in 10 per cent formalin and in a bichromate-formol solution. The tissues were sectioned at 6 μ and stained routinely with hematoxylin and eosin. Masson's trichrome stain and Sudan IV were employed when indicated.

Results

ANATOMICAL CHANGES IN THE KIDNEYS

Stock Diet Plus Serine

Rats on the stock diet receiving serine by stomach tube showed a slight reduction in the expected weight gain during the second week of the experiment, but otherwise no change was noted during life. At autopsy there was no evidence of gross abnormality in any of the animals, and the organs were of their expected weight.

In those animals sacrificed 24 hours after receiving their initial supplement of serine by stomach tube, acute renal necrosis was noted at the junction of the cortex and medulla (Fig. 1). The tubular epithelium in this area was almost completely necrotic, although the general pattern of the renal architecture was maintained. The interstitial tissue, blood vessels, and glomeruli were spared for the most part. Necrosis in most instances was complete, but an occasional tubule could be seen which was not altered structurally.

The renal lesions seen in animals sacrificed after their third supplement of serine did not differ markedly from those in the rats examined on the first day. The rats killed on the seventh day, however, presented kidneys almost entirely devoid of necrotic tissue, the degenerated tubular epithelium having been almost completely removed (Fig. 2). The tubular cells were replaced by elongated and flattened cells which formed the lining epithelium of greatly dilated tubules. Removal of the

necrotic tissue was thorough in the kidneys of animals sacrificed after their eleventh supplement of serine, so that only a very occasional fragment of degenerated tubular epithelium could be found.

The kidneys of the animals which were allowed to continue on the stock diet for 9 days following the last administration of serine showed almost complete repair, small atrophic tubules surrounded by active fibrous tissue cells and an occasional dilated tubule being the only remaining evidence of antecedent renal injury. Animals sacrificed 19 days after serine was withdrawn showed further evidence of repair in the kidneys, only a very occasional dilated tubule being seen, together with small areas of scarring containing small atrophic tubules.

Experimental Diet Alone

Animals maintained on the experimental diet failed to gain weight in the normal fashion but at autopsy presented no gross pathological lesions. The microscopical findings in the kidneys were not striking. Rats sacrificed between the eighth and twentieth days of the experiment showed minimal cellular changes in the tubules. Intracytoplasmic hyalinization and fatty degeneration were seen in certain of the convoluted tubules, and careful study revealed chromatolysis progressing to complete necrosis in certain instances. These changes were not seen in every animal, and in no instance were they pronounced.

Two of the animals sacrificed after having been on the experimental diet for a period of 20 days showed, in sections stained with hematoxylin and eosin, easily demonstrable lesions in the form of fine bluish granules, which had the microscopical appearance of calcium, within the tubular epithelial cells. The tubules involved in the process were surrounded by fibrous tissue which was infiltrated with mononuclear cells. Rats sacrificed on the thirtieth day of the experiment presented definite areas of calcification within the tubular epithelium, the granules having been replaced by laminated sheets of bluish staining material. One animal presented diffuse cortical necrosis in addition to the tubular calcification (Fig. 3). Of the 3 rats allowed to continue on the experimental diet for 40 days, 2 showed areas of tubular calcification, while 1 animal presented no lesion demonstrable histologically.

Experimental Diet Plus Serine

Animals placed on the experimental diet for a period of 7 days before receiving serine by stomach tube showed marked renal necrosis within 24 hours after the initial administration of the amino acid (Fig. 4). The renal injury was similar to that which followed the administration of the amino acid to animals on the stock diet but was more

severe. Necrosis was most marked in the innermost portion of the cortex, but sometimes extended toward the periphery to involve the descending portions of the proximal convoluted tubules. Following the third supplement of serine, in sections stained with hematoxylin and eosin, bluish granules appeared within the cytoplasm of the tubular epithelium, and even at this early period the deposit was extensive (Fig. 5). The picture varied somewhat in different animals, but in general was progressive. By the sixth day after the beginning of serine administration, the granules had been replaced by definite flakes of calcium (Figs. 6 and 7). In addition, the necrotic débris had been almost completely removed, and there was considerable fibroblastic activity associated with mononuclear, eosinophilic, and neutrophilic infiltration in the immediate vicinity of the renal injury (Fig. 8). From this point on the picture was that of a rapid repair, so that the kidneys of animals sacrificed on the ninth day of serine administration showed almost complete disappearance of mononuclear and polymorphonuclear cells, and very little necrotic tissue could be seen. An occasional dilated tubule was visible, and numerous small atrophic tubules were seen in the areas of scarring. In the kidneys of animals sacrificed on the fourteenth day of serine administration, numerous small, well defined scars containing areas of calcification were seen (Fig. 9). Kidneys removed from the animals sacrificed on the thirtieth day of the experiment showed a continuation of the healing process, with areas of tubular hypertrophy alternating with areas of renal scarring (Fig. 10). The picture closely resembled that of the finely granular kidney seen in various types of renal disease in man, the areas of hypertrophy and scarring being similar to those seen in the arteriolosclerotic kidney and also in chronic glomerulonephritis. The kidneys of animals sacrificed on the fortieth day of the experiment presented a similar, but more advanced, picture (Figs. 11 and 12).

Stock Diet Alone

Animals maintained on the stock diet until they had reached the desired weight showed no demonstrable renal lesion.

ANATOMICAL CHANGES IN ORGANS OTHER THAN THE KIDNEYS

Animals maintained on the experimental diet for a period of 8 days showed an increase in liver fat which varied considerably in amount. By the twentieth day of the experiment, marked fatty infiltration was seen in the liver. The livers of rats maintained on the experimental diet supplemented with serine did not differ markedly from those seen in

animals maintained on the experimental diet alone. However, the majority of the animals in group 3 which died during the first few days of the experiment showed degenerative changes in the liver cells around the central veins, associated with marked congestion in that area. In those animals which were fed the stock diet alone, or the stock diet supplemented with serine, no abnormal changes in the liver were noted.

Myocarditis, which in most instances was confined to the auricles, was present in approximately one-third of the animals. It was seen in all four groups of rats with approximately the same incidence.

Those animals which succumbed following serine administration during the early stages of the experiment showed acute capillary dilatation which in many instances had proceeded to transudation.

Discussion

Twelve rats maintained on a stock diet and receiving serine by stomach tube showed in every instance marked renal necrosis followed by rapid removal of the necrotic tissue and almost complete repair. The necrosis was extensive and developed rapidly, being seen in animals sacrificed 24 hours after their initial supplement of serine. The process of repair was also remarkably rapid, the necrotic tissue being almost completely removed from the kidneys by the seventh day of the experiment. It is apparent further that the continued administration of serine after the initial injury does not maintain or augment the process. Kidney repair progresses rapidly and is almost complete regardless of whether serine is continued or not.

Rats maintained on an experimental diet deficient in protein and in the B vitamins developed minimal degenerative changes in the tubular epithelium which were followed by calcification. These changes were in no instance pronounced, although they were definite and occurred in the same anatomical position in the kidney as did those lesions which followed serine administration.

Animals maintained on the experimental diet supplemented with serine by stomach tube showed renal necrosis similar to that seen in the rats fed a stock diet supplemented with serine, but more extensive. In addition, there were present on the second day following serine administration bluish intracytoplasmic granules in sections of the kidneys stained with hematoxylin and eosin. Later these granules became incorporated into rather extensive calcium deposits. Here again, the continued administration of serine did not appear to augment the initial lesions induced by the first supplement of the amino acid. The renal damage appeared to be acute and progressed rapidly to its maximum

proportions. Repair was initiated early and proceeded rapidly, but in most instances the renal injury had been so extensive that the kidney could not return to normal.

It is apparent from these experiments that, coincident with renal injury, there was capillary dilatation which in some instances was followed by passage of fluid through the vessel walls. In those animals which survived the initial renal insult, the vessels appeared to regain their tone, and these rats showed no evidence of peripheral circulatory failure. The viscera of rats dying during the stage of acute renal necrosis, however, showed marked dilatation of the capillaries and hemorrhage into the tissue spaces. This was most marked in the lungs and liver but was present in all organs. Occasionally, free fluid was seen in the serous cavities. One can conclude with reasonable safety that peripheral circulatory failure was the mechanism of death in these animals.

Rats placed on an experimental diet deficient in proteins and also in B vitamins showed, in frozen sections stained with Sudan IV, a progressive increase in the amount of hepatic fat. Finally, in sections stained with hematoxylin and eosin, large vacuolated spaces could be seen within the hepatic cells. Since the experimental diet was poor in choline or choline percursors, this was expected. The addition of serine to the diet did not appear to accentuate the process, for the livers of the animals in the third group did not differ markedly in fat content from those of the animals in the second group.

A low-grade myocarditis, confined for the most part to the auricles, was present in a fairly large percentage of the animals employed in these experiments. There was no correlation between the cardiac lesions and the experimental procedure employed in the present series.

In our studies it has been shown that serine administered by stomach tube exerts an injurious effect on the kidneys of rats. Many authors who have produced renal injury with amino acids, however, have used the parenteral route. An exception is the work of Cox, Smythe, and Fishback,[11] who found that free cystine in the diet was nephrotoxic in young rats weighing 60 gm. or less, but that older rats on the same regimen failed to develop signs of renal injury. Also, other amino acids have been shown to be nephrotoxic in young rats maintained on a diet deficient in protein.[7,8] In this connection, it may be noted that in the present study older rats (approximately 100 gm.) were employed.

SUMMARY

Rats maintained on a stock diet supplemented with serine by stomach tube showed severe renal necrosis within 24 hours after the initial supplement of amino acid. The administration of serine beyond the

first few days of the experiment did not appear to augment the necrotizing process.

The injurious effect of serine on the kidneys of rats can be greatly augmented by placing the animals on an experimental diet deficient in protein and in the B group of vitamins. Those animals which were on the experimental diet supplemented with serine showed much more extensive necrosis, followed by calcification, than did those animals receiving serine as a supplement to a stock diet which was considered adequate.

Animals maintained on an experimental diet showed rapidly progressive fatty infiltration of the liver. As this experimental diet was poor in choline or choline precursors, such fatty infiltration of the liver was expected. This finding was not modified by the administration of *dl*-serine.

Mononuclear infiltration of the myocardium was seen in all groups of rats employed in this experiment and was not considered significant. Some rats maintained on the experimental diet, which were receiving serine by stomach tube, died during the experiment. The mechanism of death here appeared to be peripheral circulatory failure.

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DESCRIPTION OF PLATES

Plate 133

FIG. 1. Section of the kidney of an animal maintained on the stock diet and sacrificed 24 hours after receiving the first supplement of serine. The tubular epithelium is almost completely necrotic. Hematoxylin and eosin stain. × 100.

FIG. 2. Section of the kidney of an animal maintained on the stock diet and sacrificed after having received the seventh supplement of serine. The necrotic material has been almost completely removed. The tubules are dilated and are lined by flattened epithelial cells. Hematoxylin and eosin stain. × 100.

FIG. 3. Section of the kidney of a rat maintained on the experimental diet alone for 30 days. Cortical necrosis is diffuse and marked, and the calcium deposits are much more prominent in this animal than in other rats maintained on the experimental diet alone. Hematoxylin and eosin stain. × 8.

FIG. 4. Section of the kidney of a rat maintained on the experimental diet for 7 days and sacrificed 24 hours after the first supplement of serine. There is complete necrosis of the tubular epithelium in certain areas. Hematoxylin and eosin stain. × 100.

FIG. 5. Section of the kidney of a rat maintained on the experimental diet and sacrificed on the third day of serine administration. In addition to necrosis, extensive bluish granular deposits are seen within the tubular epithelium. Hematoxylin and eosin stain. × 100.

FIG. 6. Section through the entire kidney of an animal maintained on the experimental diet and sacrificed on the sixth day of serine administration. The granular deposit is extensive. Hematoxylin and eosin stain. × 8.

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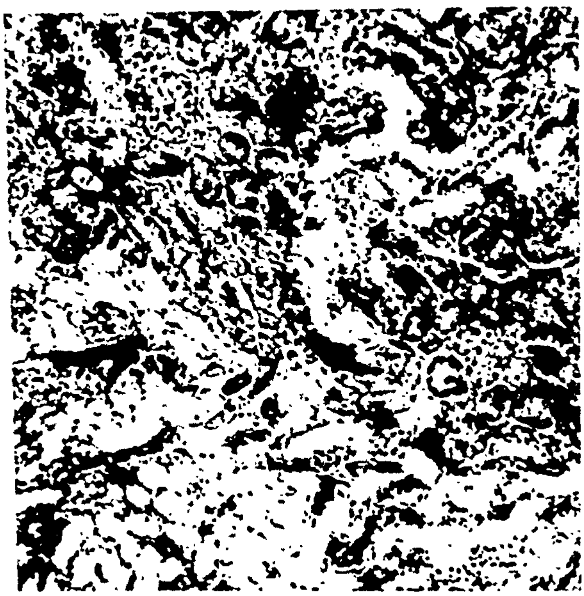

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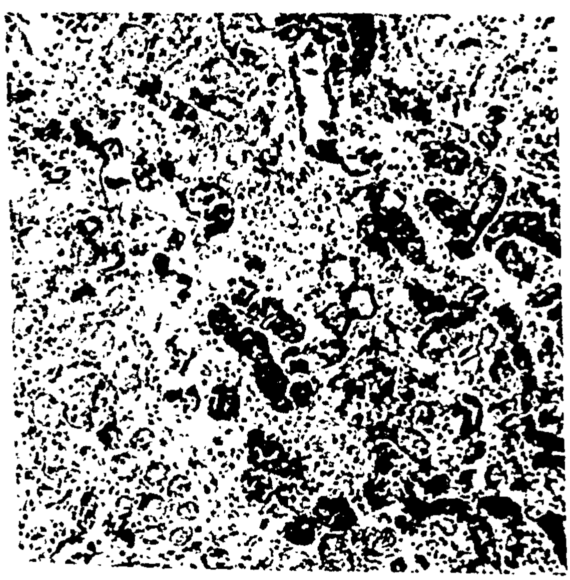

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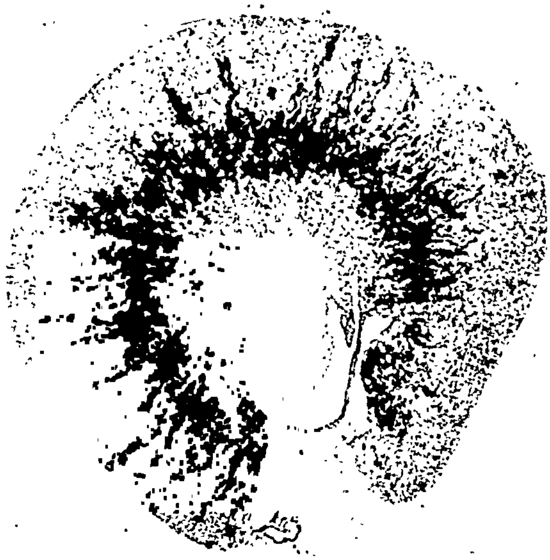

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Morehead, Fishman, and Artom

Renal Injury Following Administration of Serine

PLATE 134

FIG. 7. A higher magnification of the kidney shown in Figure 6, showing extensive cellular infiltration. Hematoxylin and eosin stain. × 100.

FIG. 8. Further magnification of the kidney seen in Figure 6. The infiltrating cells are both mononuclear and neutrophilic in type. Fibroblastic activity is marked, and a mitotic figure can be seen in the upper center of the photomicrograph. Hematoxylin and eosin stain. × 370.

FIG. 9. Section of the kidney of an animal maintained on the experimental diet and sacrificed after the fourteenth day of serine administration. The granules have become confluent. Large amounts of kidney parenchyma have been destroyed. Hematoxylin and eosin stain. × 100.

FIG. 10. Section of the kidney of a rat maintained on the experimental diet supplemented with serine and sacrificed on the thirtieth day of the experiment. Well defined calcium deposits may be seen. Hematoxylin and eosin stain. × 100.

FIG. 11. A section through the kidney of a rat maintained on the experimental diet supplemented with serine and sacrificed on the fortieth day of the experiment. Here may be seen the greatly dilated and hyperplastic tubules in the cortex and also the extensive calcium deposits and scarring at the junction of the medulla and inner stripe of the cortex. Hematoxylin and eosin stain. × 8.

FIG. 12. A higher magnification of the same section as used for Figure 11, showing the extensive destruction of the renal parenchyma and its replacement by scar tissue. Hematoxylin and eosin stain. × 100.

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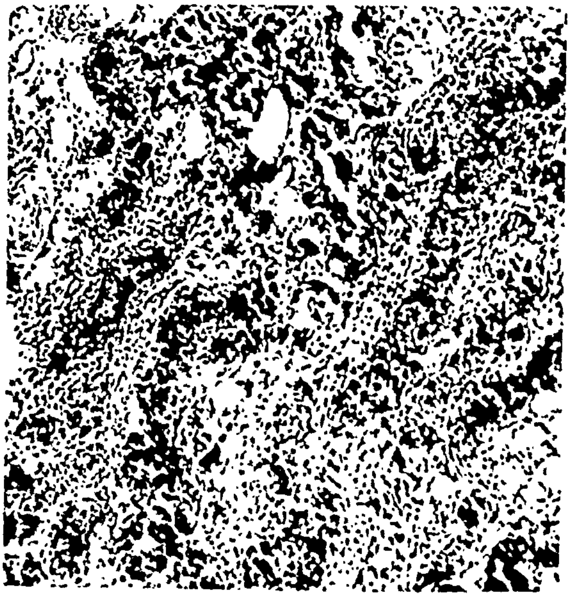

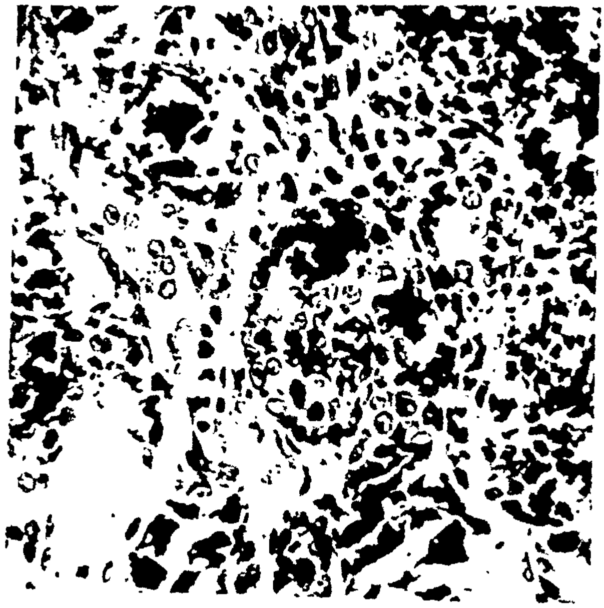
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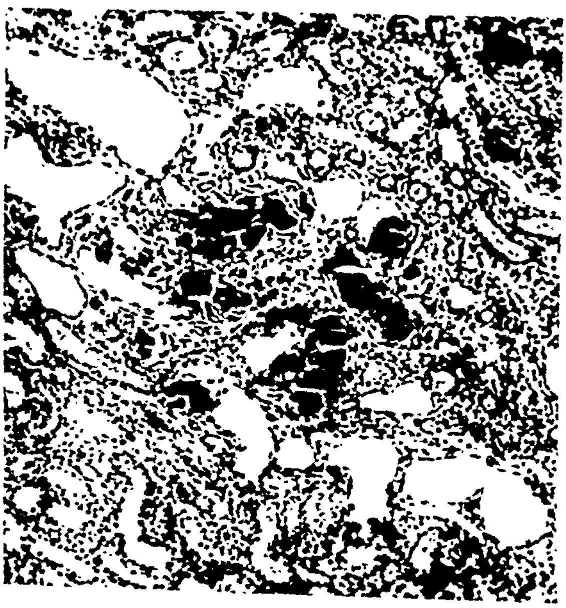

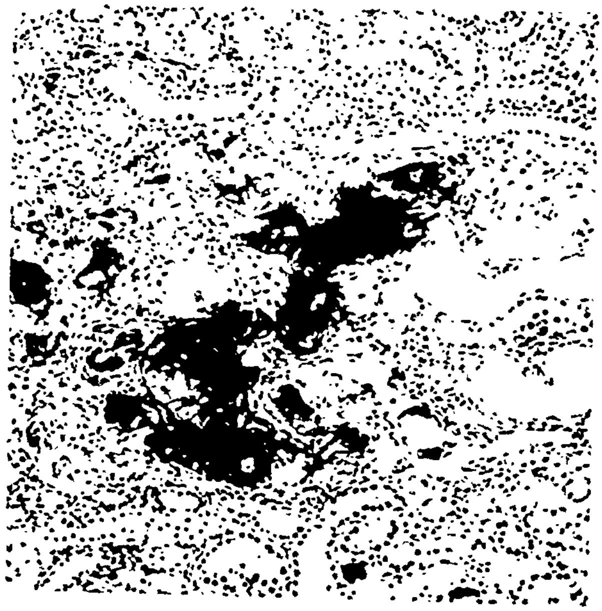
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Morehead, Fishman, and Artom

Renal Injury Following Administration of Serine

EXTRACTS FROM
MINUTES OF THE MEETING OF THE COUNCIL

THE AMERICAN ASSOCIATION OF PATHOLOGISTS AND BACTERIOLOGISTS

CLEVELAND

MAY FIFTH, 1945

THE AMERICAN ASSOCIATION OF PATHOLOGISTS AND BACTERIOLOGISTS

Extracts from Minutes of the Meeting of the Council Held at Cleveland, Ohio, May 5, 1945

Present. President CANNON, DOCTORS FORBUS, GOODPASTURE, HAYTHORN, KARSNER, MORITZ, SOULE, WARREN, and WELLER.

The following were elected to membership in the Association:

LEWIS B. BATES
BERNARD BLACK-SCHAFFER
JASPER DIXON BUSH, JR.
REUBEN CARES
ISADORE NATHAN DUBIN
KENNETH MILO ENDICOTT
HORACE KERR GIFFEN
IRVING ISRAEL GOODOF
JUNE U. GUNTER
DESMOND E. O'C. MAGNER
WILLIAM AVISON MEISSNER
HENRY D. MOON
ROBERT PAGE MOREHEAD
EMMA S. MOSS
KARL T. NEUBUERGER
AUGUSTIN R. PEALE
GEORGE G. PROSKAUER
ENID KATHLEEN RUTLEDGE
HANS G. SCHLUMBERGER
HENRY SIEGEL
RUTH SILBERBERG
WRAY JOSEPH TOMLINSON
MAX WACHSTEIN
LOUIS JENRETTE ZELDIS

At their request, Drs. Arthur L. Amolsch, Averill A. Liebow, J. H. Fisher, and Bjarne Pearson were reinstated to membership.

It was voted to accept with regret the resignations of Drs. E. H. Hatton, J. W. Jobling, M. Lederer, H. F. Traut, H. R. Churchill, and E. C. Rosenow.

The deaths of the following members were recorded with deep regret: John Shaw Dunn, Louis A. Julianelle, Frank P. McNamara, Seaton Sailer, and Grover C. Weil.

Dr. Malcolm H. Soule was reelected Associate Editor of *The American Journal of Pathology* for a term of one year.

Dr. H. M. Zimmerman, whose term expires as a member of the Editorial Board of *The American Journal of Pathology,* was reelected for a term of six years.

Dr. Cannon renewed the invitation of the University of Chicago to meet there in 1946. It was voted that, government regulations permitting, the annual scientific sessions of the Association will be held at

the University of Chicago in the spring of 1946. It was voted to adhere to the original intention of the Council and to have as the topic of the symposium, "Infectious Granulomas, Exclusive of Tuberculosis and Syphilis." Dr. Wiley D. Forbus, Professor of Pathology of Duke University, will be the referee.

It was voted to nominate Dr. Howard T. Karsner as representative of the Association in the Division of Medical Sciences of the National Research Council to succeed Col. Esmond R. Long at the expiration of his term in June, 1945.

HOWARD T. KARSNER, *Secretary*

THE AMERICAN JOURNAL OF PATHOLOGY

VOLUME XXI SEPTEMBER, 1945 NUMBER 5

EXPERIMENTAL STUDIES IN CALCIFICATION

I. THE EFFECT OF A RACHITOGENIC DIET ON THE DENTAL TISSUES OF THE WHITE RAT *

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(From the Department of Research of School of Dentistry of Loyola University, Chicago College of Dental Surgery, and the Department of Histology, University of Illinois College of Dentistry, Chicago, Ill.)

The purpose of this study was to investigate the effect of a rachitogenic diet high in calcium but low in phosphorus and deficient in vitamin D upon the dental structures of the albino rat, and thus to establish a basis for subsequent experiments on the influence of parathyroid hormone, vitamin D, starvation, and injections of phosphate upon the dental tissues of the rachitic rat. This study was further indicated by conflicting findings reported in the literature regarding the occurrence of hypoplasia of the enamel in rickets.

REVIEW OF THE LITERATURE

While there are many reports on dental changes in experimental rickets (Erdheim,[1] Fleischmann,[2, 3] Gottlieb,[4] Bauer,[5] Mellanby,[6] and others), only investigations based on experimental diets comparable to the one used in this study, *e.g.*, diets high in calcium, low in phosphorus, and deficient in vitamin D, will be given primary consideration. Karshan and Rosebury[7] reported normal formation and calcification of the enamel and only slight and regional disturbances in the calcification of the dentin. They found an increased thickness of predentin only in the basal portion of the incisor. In the molars, wide predentin was confined chiefly to the roots and pulpal floor. Similarly, Lobeck[8] found in severe rickets only globular dentin and no changes in the enamel of the incisors. Becks and Ryder,[9] on the other hand, reported hypoplasia of the enamel in animals that had been on the rachitogenic diet for 4 weeks. Table I summarizes the data of the experimental studies on rickets.

* Received for publication, September 5, 1944.

Material and Methods

This study is based on material obtained from 33 white rats that were placed on a rachitogenic diet for a period of 1 to 56 days after weaning (at 21 days) (Table II). In addition, a study was made of 25 litter-mates, 21 to 77 days of age, that were fed a normal control diet. The Steenbock-Black[10] diet no. 2965, modified by the substitution of corn meal for whole yellow corn, was used. The experimental animals gained less weight than did the controls.

Table I

Data of Previous Experiments with Rachitogenic Diets Compared with Those of Present Investigation (High Calcium, Low Phosphorus, and Deficient in Vitamin D)

Author	Diet	Number of animals	Age at beginning	Duration of experiment	Histologic Findings		
					Alveolar bone	Incisor	
						Dentin	Enamel
			(days)	(days)			
Karshan and Rosebury[7]	Steenbock and Black No.2965, modified by Epstein	?	33–41	38–57	Poorly calcified	Globulated predentin, apically	Not investigated
Lobeck[8]	McCollum No. 2638	12	0–28	18–126	Not investigated	Globulated	No changes
Becks and Ryder[9]	McCollum No. 3143	21	25	7–42	Poorly calcified	Poorly calcified	Hypoplasia
Weinmann and Schour	Steenbock and Black No. 2965 (modified)	33	21	1–56	Poorly calcified	Poorly calcified	No changes

The animals used in this and the subsequent studies were kindly made available by Drs. F. C. McLean and W. Bloom.

Table II

Summary of Histologic Findings in the Upper Jaw of 33 Rats on Rachitogenic Diet

Length of experiment	No. of rats	Incisor Average width*			Molar predentin	Cementoid		Cyst of enamel organ
		Predentin	Calcified dentin	Total dentin		Molar	Incisor	
(days)		(microns)	(microns)	(microns)				
1–4	7	15	111	126	Normal	Normal	Normal	None
5–6	6	18	112	130	Normal	Normal	Normal	+ (1)
7–8	3	23	97	120	Normal	Normal	Normal	None
21–56	17	64	70	134	2 to 3 times normal width	+ +	+ (13)	+ (8)

Numbers in parentheses indicate number of animals showing the disturbance.
* Measured at the level of completed enamel matrix (labial side).

*Histologic Preparation.** The heads of the experimental and control rats were removed and fixed in 10 per cent formalin immediately after sacrifice. The jaws were dissected and the upper incisors and molars were prepared for decalcified histologic sections. The incisors were cut in serial longitudinal sections and the molars in serial mesiodistal sections. The stains used were hematoxylin and eosin. A number of ground sections of the incisors also were prepared.

Microscopic Findings

Upper Molars

Enamel. The enamel formed during the experimental period showed no significant changes.

Dentin. The predentin showed an increase in width, so that at the end of the experiment (after 56 days on the rachitogenic diet) the predentin reached a width of 50 to 60 μ, which is about three times that of the normal animal (Table II). The greatest width of predentin was found at the floor of the pulpal chamber. The width of predentin increased from the first to the third molar.

At the beginning of the experiment, approximately two-thirds of root formation was completed in the first molar. In the third molar, root formation had just begun. The second molar showed an intermediate stage of development. Those portions of the roots which had been formed during the experimental diet consisted almost entirely of predentin (Fig. 11). The pulp and odontoblasts showed no significant changes.

Cementum. The newly formed layer of cementum (precementum) did not calcify and was considerably wider than in the normal animal. The sum of the thickness of cementoid (or precementum) and calcified cementum was the same as in normal control animals, indicating a normal rate of deposition. Resorption of the cementum was much more frequent in the experimental animals (58 per cent) than in normal animals (12 per cent). The resorption areas were localized to the cervical half of the distal root surfaces. They were shallow but might extend into the dentin.

Upper Incisor

Enamel. The enamel of the incisor showed no disturbance in its formation, maturation, or pigmentation. Hypoplasia of the enamel in the sense of a lack of enamel structure was not observed.

The *enamel organ* was normal in the basal half of the tooth. In the

* Dr. H. Pitluck assisted in the technical part of this study.

incisal portion, however, cysts were found in about 50 per cent of the animals which had been on the experimental diet for 4 weeks. These cysts developed between the inner and outer enamel epithelium and probably originated by proliferation from the stratum intermedium (Fig. 1). They remained attached to the enamel surface in a restricted area and extended basally as a result of the eruptive movement of the tooth (Figs. 2 to 4). In some cases connective tissue had penetrated the degenerating lining of the cyst and filled the entire cavity (Fig. 4). In severe cases compression and degeneration of the enamel organ occurred as a consequence of the narrowing of the periodontal space.

Dentin. In the first week of the experiment the only effect was the appearance of a darkly stained incremental band in the enamel-covered dentin. This calciotraumatic line was seen 24 hours after the beginning of the experiment (Fig. 5), and shifted gradually away from the pulp in accordance with the continued apposition of dentin (Figs. 6 to 8). The line was more distinct in the middle and in the incisal third than in the basal third. The experimental dentin showed slightly less homogeneous calcification (interglobular dentin) than the pre-experimental dentin. This change was more pronounced also in the enamel-covered dentin than in the cementum-covered dentin.

Predentin. Beginning with the second week of the experiment, both the formation (Table II) and the calcification of the dentin were markedly retarded. The retardation of calcification was more pronounced than that of formation and as a consequence the width of the predentin in the basal third sometimes increased to four times the normal width (measured at the level of the completion of the enamel matrix) (Table II). The layer of predentin was thickest in the basal and narrower in the central and incisal portions. The zone of intermediate dentin (Schour and Rogoff [11]), which in normal animals was found in the middle third of the labial dentin, was absent in two-thirds of the animals after the third week of the experiment. The basal end of the dentin which was formed by the most recently differentiated odontoblasts tended to remain entirely uncalcified (Figs. 9 and 10, basal predentin) and varied in length from 0.35 mm. to 1.8 mm. It was always longer on the concave than on the convex side.

In 6 advanced cases the lingual basal predentin did not show a gradually increased width in the anterior direction but was abruptly demarcated from the more anterior and partly calcified dentin by a step in the pulpal outline (Fig. 10) as if the odontoblasts underwent a sudden spurt of increased activity at the time and place of initial calcification of dentin.

Vascular inclusions in the dentin were absent in animals that were on

the rachitogenic diet 3 weeks or less but were present in 6 of 15 rats that were on the rachitogenic diet for a longer period.

Differences in Reaction in Labial and Lingual Dentin. The differences in the behavior of the enamel-covered and the cementum-covered dentin were enhanced after the second week of the experiment. In the labial dentin the globules were large and remained separated from each other by interglobular dentin. In the lingual dentin the globules were small and irregular and the interglobular dentin was reduced to a minimum. It showed a characteristic striation.

Rate of Apposition. The formation of the dentin was appreciably retarded in experimental rickets so that the dentin was thinner than normal. The increase of the cross section of the tooth, however, which depends on epithelial and pulpal proliferation, continued at a normal rate. The result was a disharmony between the increase in the diameter of the tooth and increase in the thickness of its dentinal walls (Fig. 12, A and B).

Cementum. The calcified layer of the cementum of rachitic animals was very thin and of normal width. The cementoid layer, however, was thickened and contained some cells (Table II). The evenly spaced dental fibers of the periodental membrane were anchored in the cementoid as Sharpey's fibers and gave it a striated appearance.

Discussion

Differences in the Reactivity of the Calcified Structures. The disturbances of the calcified dental tissues in experimental rickets were confined to the dentin and cementum. The formation of predentin was retarded and the calcification of the cementum and the dentin was impaired. When a part of the predentin formed during the experiment calcified, the calcification was incomplete, as shown by the persistence of large areas of interglobular dentin. The layer of predentin increased in width. The calcification of the newly formed cementum either was lacking or considerably restricted.

The enamel seems to be entirely immune to the metabolic disturbances of the rickets produced by our experiment. Not only is the formation of enamel undisturbed, but the maturation (calcification) of the enamel proceeds normally. The difference in the behavior of the enamel from that of the other hard tissues (dentin, cementum, and bone) may be caused by the chemical difference of the matrix of these different tissues. No principal differences exist in this respect between bone, cementum, and dentin, all mesodermal tissues. The enamel matrix, however, as an epithelial product, shows tinctorial peculiarities, at least, which indicate a different chemical composition.

The rachitic changes in bones are more complex because, in addition to the disturbance in calcification, there are the secondary changes which are caused by the resistance of uncalcified cartilage and bone to resorption and which are entirely lacking in the dental tissues. Resorption constitutes an essential phase in the development and growth of bones but plays no part in the development and growth of the incisors and molars of the rat.

Retardation in the Calcification of the Dentin. In rickets the predentin is significantly wider in the apical third but tends to be approximately of normal width in the middle and incisal thirds.

Since, with the continuous eruption of the incisor, the basal third of the dentin eventually moves anteriorly into the middle portion of the tooth, and since in that portion of the tooth calcification tends to be normal, the disturbance in calcification must be one of retardation in the rate of calcification. The retardation in the rate of calcification of dentin is further indicated by the fact that the predentin is not only wider, but persists longer, than normally, especially at the lingual side (basal predentin).

Hypoplasia of Enamel. The absence of hypoplasia of the enamel in our material confirms the findings of Karshan and Rosebury [7] and of Lobeck.[8] The cystic degeneration of the enamel organ was confined to the incisal half of the incisor where the enamel had already been completed in its formation and calcification, and therefore should not be considered as an enamel defect (Gottlieb [4]).

Several authors [12-15] have found hypoplasia of the enamel in 20 to 25 per cent of cases of rickets, while Fleischmann,[2, 3] whose findings were based on histologic and post-mortem evidence, found no relationship between hypoplasia of the enamel and rickets.

It seems that rickets *per se,* which results from a diet high in calcium, low in phosphorus, and deficient in vitamin D, is not responsible for hypoplasia of the enamel.

Summary and Conclusions

This investigation was based on a study of the teeth of 33 white rats which were placed after weaning on a rachitogenic diet high in calcium but low in phosphorus and deficient in vitamin D for a period of from 1 to 56 days, and of 25 litter-mate controls.

The histologic findings were:

1. Enamel formation and calcification were normal. Hypoplasia of the enamel was absent. The enamel organ showed cystic degeneration in the incisal half of the incisor.

2. Dentin formation was retarded in rate. Calcification of dentin was

retarded and also disturbed. A calciotraumatic line was the immediate response which marked the beginning of the experiment. The newly formed dentin showed an interglobular texture. The enamel-covered dentin was more severely affected than the cementum-covered dentin.

3. Cementum formation was normal in rate but its calcification was defective. Shallow areas of resorption on the molar roots were more frequent than in normal animals.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 135

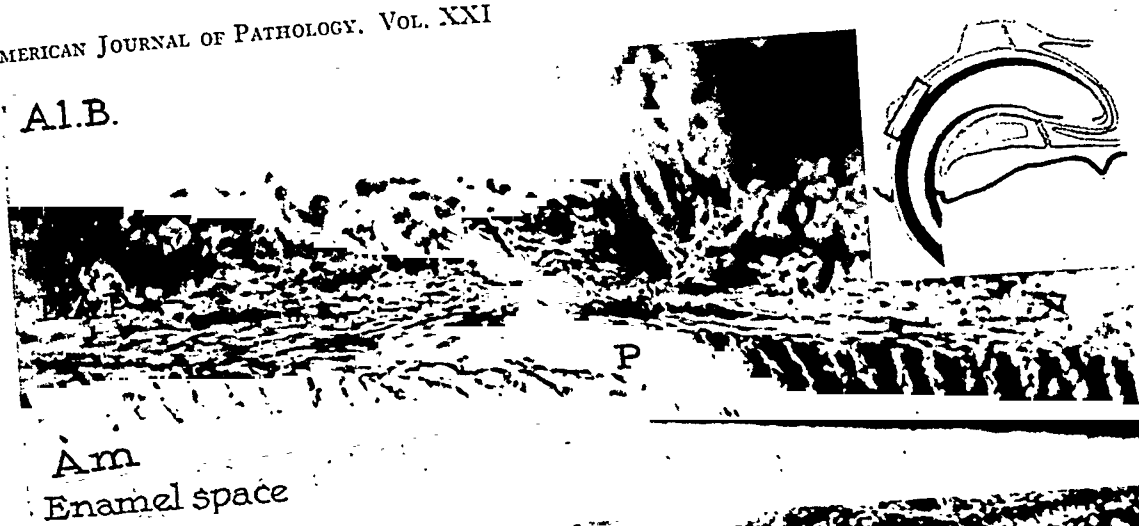
Photomicrographs of decalcified, longitudinal, midsagittal sections of the labial anterior region of the upper incisor of rats weaned and placed on a rachitogenic diet at 21 days of age. The area is indicated in the insert in Figure 1. Al.B. = labial alveolar bone; Am. = ameloblasts. The space next to the ameloblasts was originally occupied by enamel which is lost during decalcification. All illustrations were prepared from sections stained with hematoxylin and eosin.

FIG. 1. Rat 404, 50 days old. Section shows solid proliferation (P) of the stratum intermedium and outer enamel epithelium and the normal smooth surface of the ameloblastic layer. L.A.P. = labial alveolar periosteum. × 119.

FIG. 2. Rat 1602, 52 days old. Multiple sites of proliferation (P) and cysts (Cy) of the stratum intermedium and outer enamel epithelium are indicated. × 88.

FIG. 3. Rat 1501, 49 days old. A single large cyst (Cy) has its wall formed by the stratum intermedium and the partly degenerated outer enamel epithelium. × 88.

FIG. 4. Rat 303, 54 days old. A large cyst is filled with connective tissue (C.T.), which has apparently penetrated through gaps (G) in the epithelium. The surface of the enamel is smooth. × 119.



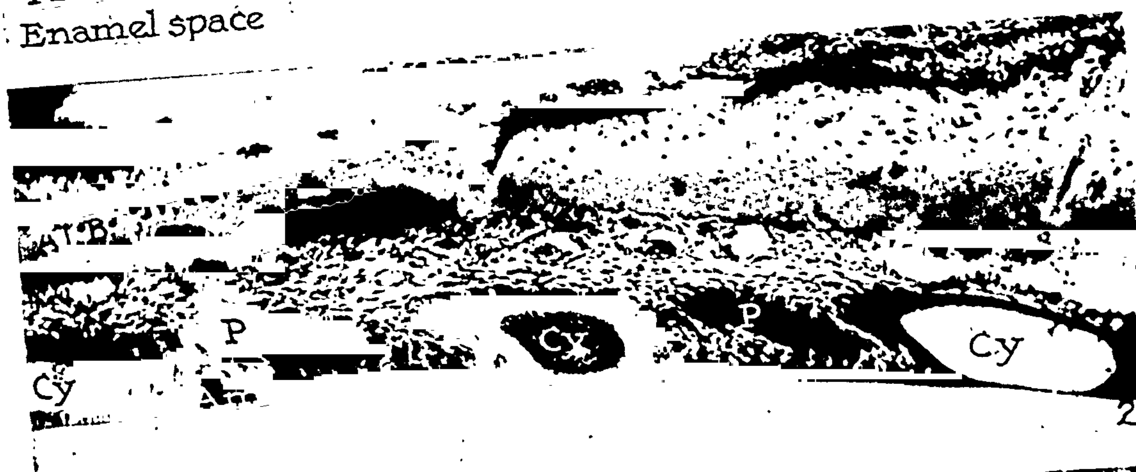


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PLATE 136

FIGS. 5–8. Photomicrographs of longitudinal sections of the labial dentinal wall from the middle third of the upper incisor. A dark-staining stripe in the dentin, the calciotraumatic line (X), represents the immediate response to the rachitogenic diet and marks the beginning of the experiment. $\times$ 102.

FIG. 5. Rat 3203, 22 days old, placed on rachitogenic diet for 24 hours. The calciotraumatic line (X) is next to the predentin.

FIG. 6. Rat 3205, 25 days old, placed on rachitogenic diet for 3 days. The calciotraumatic line (X) is 47 μ distant from the odontoblastic layer.

FIG. 7. Rat 3207, 27 days old, placed on rachitogenic diet for 5 days. The calciotraumatic line (X) is 84 μ distant from the odontoblastic layer.

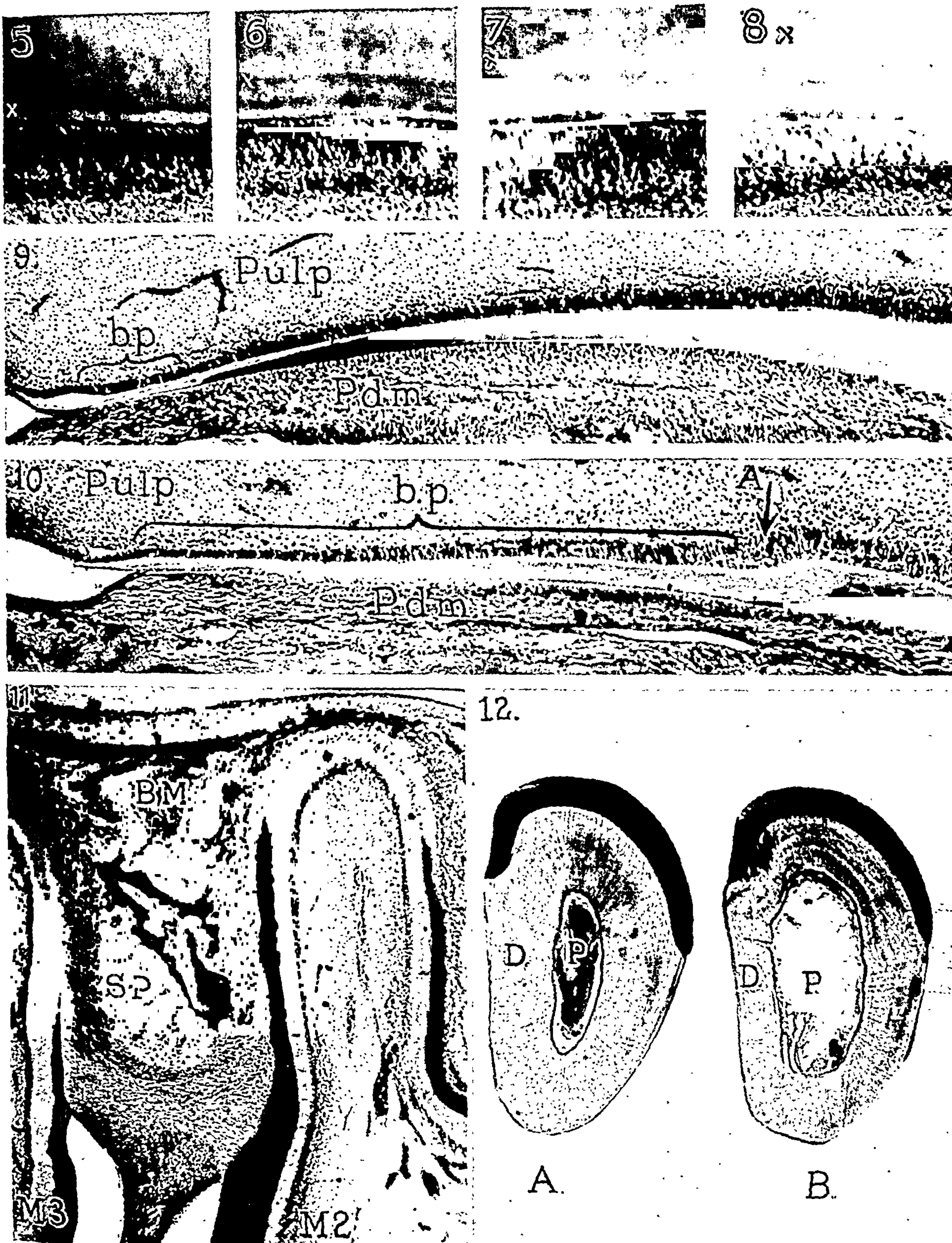
FIG. 8. Rat 3209, 29 days old, placed on rachitogenic diet for 7 days. The calciotraumatic line (X) is 112 μ from the odontoblastic layer.

FIG. 9. Photomicrograph of a longitudinal section of the lingual basal portion of the upper incisor of rat 1801, which was 67 days of age and was placed on the control basal diet after weaning at 21 days of age. Of note are the short uncalcified basal area, the basal predentin, b.p., and the smooth pulpal surface. P.d.m. = periodontal membrane. $\times$ 53.

FIG. 10. Photomicrograph of area corresponding to that of Figure 9 taken from rat 1802, which was 67 days of age and was fed the rachitogenic diet for 46 days. Here are seen the extensive uncalcified basal area (b.p.) and the sudden increase in dentin thickness at A. $\times$ 53.

FIG. 11. Photomicrograph of mesiodistal section of distal root of second (M2) and mesial root of third (M3) upper molar of rat 1902, 49 days of age, which was weaned at 21 days of age and then placed on a rachitogenic diet for 28 days. Section shows the difference in the calcification of dentin and cementum in the roots of the second and third molar, reduction of the bone marrow spaces (BM), and the eccentric location of the pre-experimental calcified core in the interdental alveolar septum (SP). $\times$ 58.

FIG. 12. Photomicrographs of transverse ground sections of the incisor of normal rat 1801 (A) and rachitic rat 1802 (B). The animals were litter-mates, 67 days old. The circumference of the teeth is essentially the same but the dentin (D) of the normal rat (A) is wider and the pulpal cavity (P) narrower than in the rachitic animal (B). $\times$ 58.



Weinmann and Schour

Effect of Rachitogenic Diet on Dental Tissues

EXPERIMENTAL STUDIES IN CALCIFICATION

II. The Effect of a Rachitogenic Diet on the Alveolar Bone of the White Rat *

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The studies of bone growth in rickets thus far reported have been concerned chiefly with disturbances in growth of endochondral bone. Reports on the rachitic disturbances in endomembranous bone, uncomplicated by the presence of growing cartilage, are lacking except for frequent statements that the bone trabeculae are characteristically bordered by osteoid (Erdheim,[1] Becks and Ryder,[2] McLean [3]). Similarly, little attention has been given to a consideration of the resorption pattern in rachitic bone. The alveolar bone of the incisor and molar regions of the albino rat may serve as a good test object in the analysis of both apposition and resorption, since this bone is undergoing relatively rapid and continuous architectural reconstruction coincident with the physiologic movement of the teeth. In addition, the bone is always in a relatively constant relationship to the tooth which serves as a ready point of reference. An analysis of the alveolar bone, therefore, promises to be of special significance for the understanding of rachitic bone changes in general.

Material and Methods

The material and methods were the same as those used for the preceding paper (Table I).[4] Thirty-three white rats † were placed on a rachitogenic diet for a period of 1 to 56 days after weaning (at 21 days). In addition, a study was made of 25 litter-mates, 21 to 77 days of age, fed on a normal control diet. The Steenbock-Black [15] diet no. 2965, modified by the substitution of corn meal for whole yellow corn, was used.

The heads of the experimental and control rats were removed and fixed in 10 per cent formalin immediately after sacrifice. The jaws were dissected and portions bearing the upper incisors and molars were prepared for decalcified histologic sections. The incisors were cut in serial longitudinal sections and the molars in serial mesiodistal sections. The sections were stained with hematoxylin and eosin.

Microscopic Findings

A description of the findings in the experimental animals will be preceded by a brief presentation of the normal histologic structures.

* Received for publication, September 5, 1944.

† The animals used in this series of studies were kindly made available by Drs. F. C. McLean and W. Bloom.

A. UPPER MOLAR REGION

Normal Animals

The alveolar bone (Text-Fig. 1-A and Fig. 1), as seen in mesiodistal section of the upper molar, consists of (1) a thin, compact basal plate which forms part of the boundary of the temporal fossa and part of the alveolar wall of the apices of the roots, and (2) the spongy interdental and interradicular septa. The periodontal borders of the septa tend to be continuous. The septum between the first and second molars assumes a triangular form. Its gingival half is dense and contains small marrow spaces that are filled with loose connective tissue. Its basal half contains larger intercommunicating spaces that are filled with red marrow.

The alveolar process in the bifurcation of the roots presents a spongy framework of thin bone trabeculae. It consists of a large central portion which is filled with red marrow, and a relatively narrow peripheral portion. The latter borders the periodontal membrane and contains loose connective tissue in its marrow spaces.

The osteoblasts are numerous at the crest of the interradicular and interdental septa. They are less prominent in the small bone-marrow spaces near the periodontal membrane. Osteoclastic resorption is seen frequently at the endosteal surfaces of the bone trabéculae.

Corresponding to the distal drift of the molars, bone apposition can be seen on the mesial alveolar walls, bone resorption on the distal walls. An exception is the alveolus of the mesial root of the first molar. The divergence of this root from the other roots of the first molar necessitates resorption at the mesial wall during the tipping occluso-distal movement of this tooth. The drifting movement of the rat molars continues throughout the life of the animal. It is, however, intermittent. During the rest periods thin layers of bone are apposed in small restricted areas upon the resorbed bone and the principal fibers of the periodontal membrane are reattached to the alveolar wall (Sicher and Weinmann [5]).

The bone trabeculae stain intensively with hematoxylin, suggesting normal calcification. Osteöid borders are almost entirely missing. Only an occasional extremely delicate eosin-staining seam is found at the crest of the interdental and the interradicular septa.

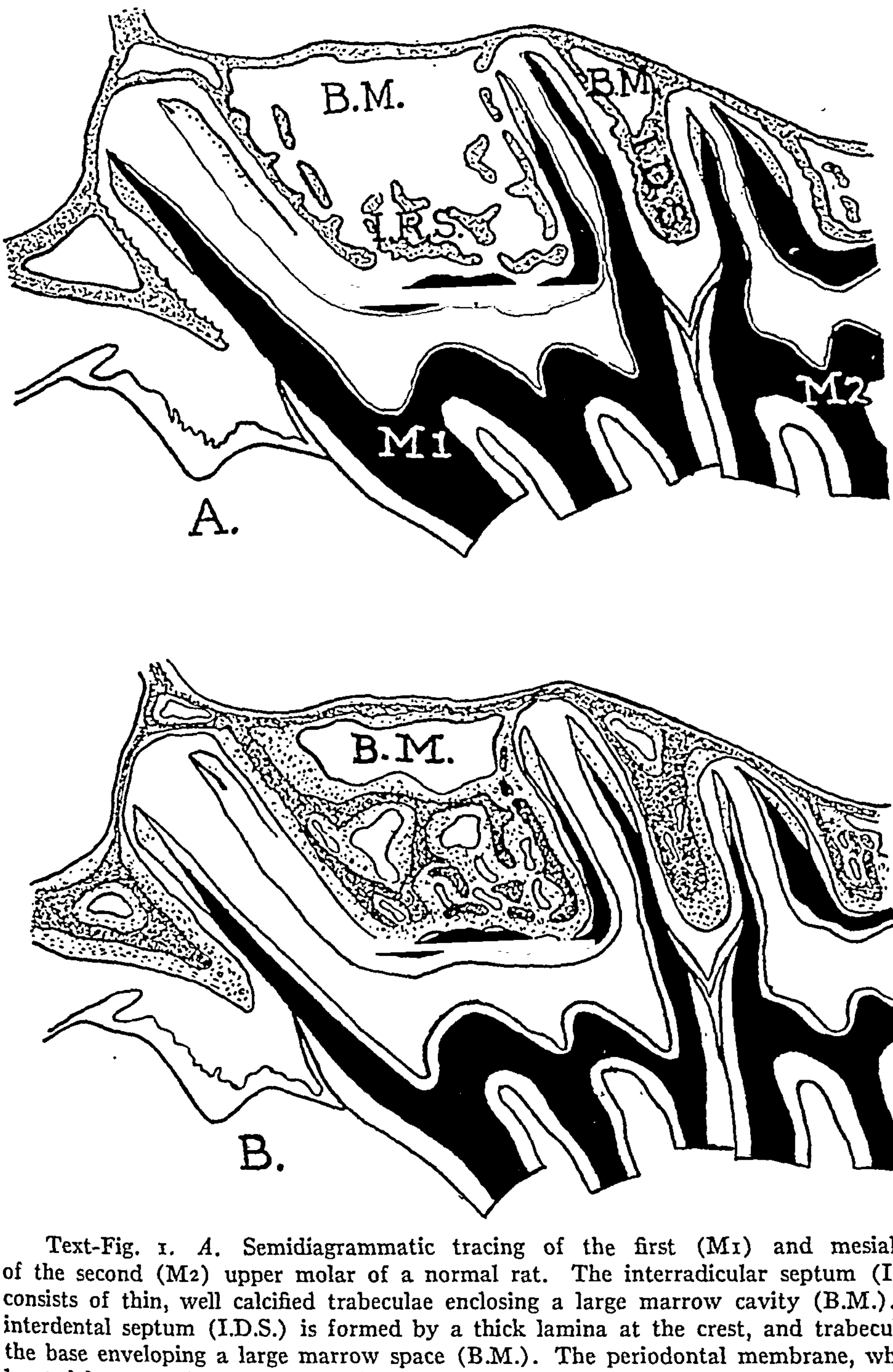
The *periodontal membrane* is of fairly uniform width.

Experimental Animals

Alveolar Bone. The alveolar bone presented a normal picture (Fig. 1) until the fifth day on the rachitogenic diet. At this time the alveolar crest showed an osteoid seam which (Table I) continued to increase in thickness throughout the duration of the experiment (Text-

Fig. 1 and Fig. 2). The maximal width of the osteoid tissue was not greater than the expected apposition of bone in a given area (Hoffman and Schour [6]).

As a result of the distal movement of the molars (Sicher and Wein-



Text-Fig. 1. *A*. Semidiagrammatic tracing of the first (M1) and mesial half of the second (M2) upper molar of a normal rat. The interradicular septum (I.R.S.) consists of thin, well calcified trabeculae enclosing a large marrow cavity (B.M.). The interdental septum (I.D.S.) is formed by a thick lamina at the crest, and trabeculae at the base enveloping a large marrow space (B.M.). The periodontal membrane, which is located between the alveolar bone and the roots, is of fairly even width. *B*. Semidiagrammatic tracing of same region as shown in *A*, but taken from a rachitic rat. The trabeculae are increased in thickness and the marrow spaces (B.M.) are decreased in size by apposition of osteoid tissue (lighter stippled areas). The periodontal membrane is reduced in width and for the most part obliterated at the crest of the interradicular septum.

mann [5]) the mesial alveolar wall showed apposition of osteoid tissue which gradually increased in thickness. The distal alveolar walls, on the other hand, showed chiefly progressive resorption, apposition of osteoid tissue occurring only in a few restricted areas. In the area of the first molar, because of the divergence of its roots, apposition of osteoid tissue was found at the distal, and resorption at the mesial, alveolar wall of the mesial root.

The trabeculae of the spongy bone in the alveolar septa were covered also by a layer of osteoid tissue which increased in thickness with the duration of the experiment. This proceeded at the expense of the bone-

TABLE I

Summary of Histologic Findings in the Upper Molars, Incisors, and Related Supporting Tissues of 33 Rats on a Rachitogenic Diet

Length of experiment	No. of rats	Osteoid alveolar bone			Ankylosis of periodontium of molar	Reduction of labial alveolar periosteum
		Molar	Incisor			
			Ling. plate	Lab. plate		
(*days*)						
1–4	7	N	N	+	N	N
5–6	6	?	N	+	N	+ (3)
7–8	3	+	+	+	N	+ (3)
21–56	17	+++	+++	+++	+ (10)	+ (4) ++ (11)

Numbers in parentheses indicate number of animals showing the disturbance.
N = No recognizable deviation from normal.
+ = Recognizable deviation from normal structure or the presence of the abnormality when specifically indicated.

marrow spaces (Fig. 2). Osteoclastic resorption, which was characteristic in the control animals, was almost entirely absent in the osteoid-covered trabeculae.

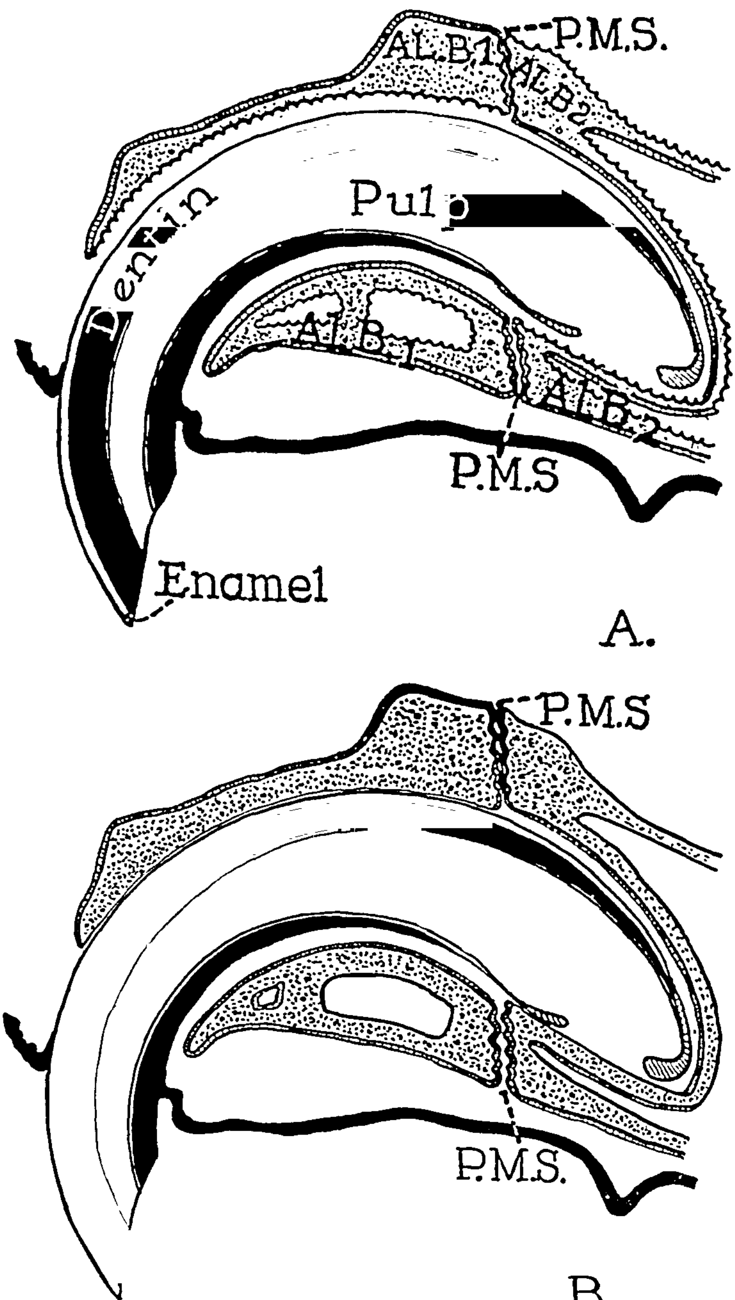
Periodontal Membrane. The first changes in the periodontal membrane occurred after the third week of the experiment. The periodontal space was narrowed and the periodontal tissues were compressed at the sites of physiologic bone apposition. The changes were most severe in the region of bifurcation at the height of the interradicular septa. Complete obliteration of the periodontal space (Fig. 2) occurred in this area in 10 of 17 cases (Table I). At the periapical region the width of the periodontal membrane remained normal.

B. UPPER INCISOR REGION

Normal Animals

The alveolar bone of the upper incisor is divided into an anterior premaxillary and a posterior maxillary portion by the premaxilla-maxillary

suture (Text-Fig. 2-A). The suture runs in an almost vertical plane. The anterior portion constitutes the anterior two-thirds of the labial bone and almost the entire lingual alveolar bone. The posterior portion



Text-Fig. 2. *A*. Semidiagrammatic tracing of the upper incisor of a normal rat. The alveolar bone consists of the anterior (Al.B. 1) and posterior portions (Al.B. 2) which are separated by the premaxilla-maxillary suture (P.M.S.). Scalloped borders indicate sites of resorption; double bars with crossbars indicate sites of apposition (see Table II). *B*. Semidiagrammatic tracing of the upper incisor of a rachitic rat, showing absence of sites of resorption. The width of the periodontal membrane is reduced or the membrane partially absent in regions where resorption normally occurs (see Table II).

constitutes the fundus and the posterior third of the labial alveolar bone. The labial alveolar bone consists of a thin premaxillary plate and a maxillary portion which serves as a thimble in which the basal end of the incisor rests.

The lingual alveolar bone (Text-Fig. 2 and Fig. 3) is sickle-shaped in longitudinal section. It consists of a periodontal plate, which parallels the curvature of the concave surface of the incisor, and a somewhat thicker periosteal plate which parallels and faces the palate. Both

TABLE II

Sites of Apposition and Resorption in Alveolar Bone of Upper Incisors of Normal Rat and Sites of Apposition in Rickets

Divisions of alveolar bone	Subdivisions		Normal: Sites of Apposition	Normal: Sites of Resorption	Rickets*: Sites of Apposition
Anterior or premaxillary portion of alveolar bone	Anterior two-thirds of labial alveolar bone	Periosteal	+		+
		Alveolar		+	−
	Lingual alveolar bone	Periodontal	+		+
		Endosteal		+	−
		Palatal	+		+
Posterior or maxillary portion of alveolar bone	Posterior third of labial alveolar bone	Alveolar	+		+
		Nasal		+	−
	Fundus	Nasal	+		+
		Alveolar		+	−

* Resorption is inhibited in rickets.

plates converge incisally at an acute angle at the lingual alveolar crest and are joined by a few transverse trabeculae. The lingual alveolar bone contains in its center spaces filled with islands of red marrow that are embedded in fatty marrow. A blind recess of the maxillary sinus extends into the posterior portion of this bone. The presence of an extremely thin osteoid border at the periodontal surface indicates relatively fast apposition of bone at this site. Howship's lacunae and osteoclasts are present occasionally along the endosteal surfaces.

The fundic bone is extremely thin. It is bordered on its nasal surface by a layer of mucous glands of the maxillary sinus (Fig. 10).

Table II gives the normal sites of apposition and of resorption in the alveolar bone of the upper incisor region.

Periodontal Membrane. The periodontal membrane, as a specialized connective tissue, may be classified into three portions:

1. The Periodontal Membrane Proper. The periodontal membrane

proper is confined to the concave and lateral cementum-covered surfaces of the incisor and serves as a suspensory ligament. In adaptation to the continuous eruption of the tooth, this ligament is arranged in three distinct layers (Fig. 5): the alveolar fibers, the dental fibers, and the intermediate plexus. The alveolar and dental fibers are arranged in a radial direction, *i.e.*, at right angles to the surface of the tooth. The alveolar fibers form strong bundles between which vessels and nerves are contained in loose connective tissue. The vessels are near the bone surface and run at right angles to the long axis of the tooth. The dental fibers form an almost continuous layer.

2. The Labial Alveolar Periosteum. The labial alveolar periosteum is confined to the convex side of the tooth (Fig. 7). It lies between the enamel organ and bone and consists of dense connective tissue, the fibers of which run parallel to the surface of the bone. The tissue is highly vascularized, with most of the vessels running close to the surface of the bone and parallel to the long axis of the tooth. Corresponding to the bones which form the alveolus at the convex side of the tooth, the labial alveolar periosteum consists of a premaxillary and maxillary portion.

3. The Fundic Portion. The fundic portion lies between an epithelial diaphragm and the fundic bone and is differentiated into the hammock ligament upon which the basal ends of the incisors rest and into a layer of very loose connective tissue which is adjacent to the tooth (Fig. 10).

Experimental Findings

Alveolar Bone. The reaction of the alveolar bone was different in the normal sites of bone apposition and in the normal sites of bone resorption.

In the normal sites of bone apposition the new formation of bone proceeded at a fairly normal rate but the calcification of the newly formed matrix was lacking. Here an increase in the width of osteoid tissue was observed beginning with the second week of the experiment (Text-Fig. 2 and Table II). Apposition of osteoid tissue (Table I) also occurred along the trabeculae of the spongy bone, especially in the lingual alveolar plate (Figs. 3 and 4). The gradual thickening of the trabeculae led to a narrowing of the marrow spaces.

In the normal sites of bone resorption (Text-Fig. 2) resorption proceeded until the calcified bone had been removed and the remaining bone consisted entirely of osteoid tissue when resorption no longer occurred. The bone now gradually increased in thickness because the continued apposition was not balanced by simultaneous resorption (Figs. 7 to 11).

Immediately anterior to the premaxilla-maxillary suture (Text-Fig.

2) the thin labial alveolar plate was connected with the frontal process of the premaxilla by a number of vertical bone trabeculae. These trabeculae were normally covered by osteoblasts. During the fifth week of the experiment they showed in many places wide seams of osteoid tissue, whereas their core consisted of well calcified bone (Fig. 12). When the resorption, proceeding at the periodontal surface of the premaxilla, reached this system of trabeculae, the different reaction of osteoid tissue and bone could be observed. Osteoclasts were seen attacking the calcified core of the trabeculae, whereas the osteoid tissue did not show signs of osteoclastic resorption (Fig. 13).

The Periodontal Membrane. The periodontal membrane proper, at the lingual or cementum-covered side of the tooth, remained, as a rule, normal in width. A narrowing of the space was observed only once. In advanced cases the arrangement of the suspensory fibers was markedly disturbed. The normal arrangement of the periodontal fibers (Fig. 5) in three layers was almost lost in the incisal portion where they followed a direct course from bone to the cementum (Fig. 6). A number of lymphoid wandering cells were found between the bundles of the principal fibers.

The labial alveolar periosteum of the tooth showed a marked difference in the premaxillary and maxillary portions of the alveolus. In the maxillary part no significant changes were seen. In the premaxillary part a gradual narrowing of the periodontal space (Table I) led to an increasing compression of the connective tissue and blood vessels (Figs. 8 and 9). In advanced stages the connective tissue underwent hyaline degeneration. The hyaline changes appeared in isolated areas as round or oval islands which later fused to form a trabeculae-like network (Figs. 8 and 9). The latter included connective tissue cells and sometimes multinucleated giant cells (osteoclasts). In its staining reaction the hyalin resembled osteoid tissue. Differentiation of the hyalin and the adjacent osteoid tissue was not always sharp. The absence of osteoblasts indicated that this tissue was not the result of apposition.

The fundic connective tissue underwent changes very similar to those of the premaxillary alveolar periosteum. The fundic space was gradually narrowed and the loose connective tissue and its blood vessels were compressed. In advanced stages the tissue adjacent to the bone underwent hyaline degeneration (Fig. 11).

Discussion

Growth of bone and its maintenance as a functioning organ is dependent upon the balanced interaction of bone apposition and bone resorption. The processes of apposition and resorption have to be

studied with equal emphasis in order to understand fully the rachitic changes of the skeleton. The alveolar bone of the rat is a useful test object for such studies. The normal relations between bone and tooth and between the growth changes of the bone and the growth changes and physiologic movement of the teeth have been sufficiently well clarified to form a basis for experimental analysis (Sicher and Weinmann [5]). The pathologic changes in alveolar bone can be studied by examining the disturbances of the relation between bone and tooth. A rachitic diet acts primarily on the bone and dentin, but does not influence the shape of the teeth (Weinmann and Schour [4]).

Our findings agree with the generally accepted view that the primary cause of the pathologic changes following rachitogenic diet is the failure in calcification (McLean,[3] Pommer,[7] Howland,[8] Dodds and Cameron [9]). The mechanism by which some of the rachitic deformities are produced is secondary and depends on the peculiar anatomy and physiology of each region, and especially on the local pattern of apposition and resorption.

A. The Spongy Bone

In the spongy bone next to the bone marrow the trabeculae thicken and the marrow spaces become narrow as a result of the slackening and final cessation of resorption. The reason for the inhibition of resorption appears to be the failure of the newly formed bone to calcify. Osteoid tissue is almost immune to resorption, as are the uncalcified cementum (cementoid) and the uncalcified dentin (predentin) (Gottlieb,[10] Orbán and Weinmann,[11] Kronfeld [12]). The resistance of osteoid tissue to resorption seems to be caused not only by its lack of calcium salts but by the lack of those changes in the organic matrix which precede or accompany calcification. That the rachitic changes in spongy bone are a result of an inhibition of resorption and not of an overproduction of new bone is indicated by the fact that osteoclasts are absent in advanced stages and that measurements at sites of regular undisturbed bone formation, as at the crest of the alveolar septa of the molars, indicate a normal rate of bone apposition. McCollum, Simmonds, Shipley, and Park [13] observed a reduction in resorption in experimental rickets. They explained this lack of resorption on the basis of changes occurring in the osteoblasts and cartilage cells.

B. The Alveolar Bone of the Molar Region

In a majority of cases an encroachment of the periodontal membrane in the region of bifurcation occurred after 3 weeks on the experimental diet. A reduction in width of the periodontal membrane was found on

the mesial side, whereas the periodontal membrane remained normal on the distal side and at the fundus. These findings may be explained on the basis of the normal growth pattern of the jaws. The occlusal movement of the molars is coordinated with, and made possible by, the increase of distance between maxillary and mandibular body as a consequence of the growth of the mandibular ramus. The latter increases in height by endochondral bone growth at the mandibular condyle. The condylar growth is in every respect the same as the longitudinal growth of the shaft of the long bone at the epiphyseal cartilage.

We know that the endochondral epiphyseal growth of bone is considerably retarded in experimental rickets (Dodds and Cameron [9]), and probably the same holds true for the condylar growth of the mandible. Thus space for the eruption of the molars cannot be provided to the normal extent in rachitic rats and the occlusal movement of the molars is therefore mechanically checked.

In the normal rat the alveolar bone of the molar shows three distinct zones of apposition (Hoffman and Schour [6]). The greatest amount of apposition occurs at the crest of the alveolar septa, less at the mesial alveolar, and least at the fundus. It has been suggested (Sicher and Weinmann [5]) that the apposition at the crest of the interradicular septa causes the vertical eruption of the molar, that the apposition of bone at the fundus is secondary to the occlusal eruption, and that the distal drift of the rat molar is caused by apposition of bone at the mesial alveolar wall. As the apposition of bone at the crest of the interradicular septum of rachitic animals proceeds, the periodontal spaces at the bifurcation of the immobilized molar is gradually narrowed and finally obliterated. Ankylosis between bone and molars in rachitic rats was reported previously (Becks and Ryder,[2] Orbán and Weinmann [11]). The progressive apposition of bone at the mesial alveolar wall narrows the mesial periodontal space of the immobilized tooth. The bone formation at the fundus of the molar is greatly inhibited, apparently because the tooth is not elevated in its socket.

C. The Alveolar Bone of the Upper Incisor Region

A marked reduction of the periodontal membrane has been observed along that part of the alveolus where resorption normally occurs, that is, the premaxillary labial portion and the maxillary fundic portion (Figs. 9 and 11). Since the reason for this physiologic resorption is different in these two areas, the mechanism of the pathologic changes will be considered separately.

The Premaxillary Labial Portion. In the premaxilla the alveolus of the normal rat is widened by resorption at the convex side of the tooth,

mainly because of the increase of the diameter of the incisor (Sicher and Weinmann [5]).

The widening of the alveolus in the premaxilla comes to a standstill in rachitic rats when the calcified bone present at the beginning of the experiment has been resorbed. Then the premaxillary alveolar plate at the convex side of the tooth consists only of osteoid tissue, which is resistant to resorption. Since the growth of the incisor in diameter progresses normally (Weinmann and Schour [4]), the tooth encroaches upon the periodontal tissues on the convex side (Figs. 8 and 9). Thus the width of the periodontal space is narrowed and later on the periodontal tissues are compressed between the growing tooth and the unyielding osteoid tissue. The impairment of the circulation of the blood in this area leads to hyaline degeneration of the connective tissue. The premaxillary periodontal space on the convex side of the incisor is finally obliterated.

The Maxillary Fundic Portion. Sutural growth between maxilla and premaxilla complicates the relation between the maxillary part of the alveolus and the teeth. If the tooth is considered as the fixed point, growth in the premaxilla-maxillary suture moves the fundic plate toward the basal end of the tooth and the maxillary alveolar plate at the convex side of the tooth away from the tooth. A reduction of the periodontal width at the fundus and an increase in the periodontal width at the convex side of the tooth would result if these tendencies were not compensated by resorption at the fundus and apposition at the maxillary alveolus on the convex side of the tooth (Text-Fig. 2). This picture of the normal animal has to be taken as a basis for the understanding of rachitic changes. In advanced rickets, the resorption of osteoid tissue fails and therefore the resorption of the fundic bone ceases. Since, however, growth in the suture continues, the periodontal width continues to be reduced (Fig. 11) and the periapical tissue may even become compressed and may undergo hyaline degeneration. The widening of the maxillary periodontal space at the convex side of the incisor by sutural growth and the apposition of osteoid tissue in those areas continue. The maxillary part of the periodontal membrane at the convex side of the tooth shows, therefore, no pathologic changes in rachitic rats.

Eruption

The eruption of the teeth in experimental rickets is retarded. Although no actual measurements of the rate of eruption have been made in rachitic animals, the histologic observations are convincing. The encroachment of the periodontal membrane between interradicular septa and the molars indicates clearly an arrest of the vertical movement of

these teeth. The retardation of the eruption of the rat incisor is indicated by the formation of a thick layer of cementoid tissue and by the disorganization of the periodontal membrane (Fig. 6). The division of periodontal membrane into three layers, which is an adaptation to rapid longitudinal eruption (Sicher [14]), disappears almost entirely. As discussed previously, the disturbance of eruption appears to be secondary to the retardation of bone growth at the mandibular condyle.

Summary and Conclusions

This histologic study is based on 33 rats which were placed on a rachitogenic diet for a period of 1 to 56 days beginning with weaning. The histologic findings were:

1. The formation of new bone seems to proceed at a normal rate but it remains uncalcified and persists as osteoid tissue.
2. Osteoid tissue fails to undergo resorption.
3. The consequences of the failure of resorption of osteoid tissue are:

a. Excessive accumulation of osteoid tissue. The sites which normally show apposition continue to do so while the sites which normally show resorption are inactive.

b. Distortion of the growth pattern of the alveolar bone, since the normal growth pattern depends upon a balance between apposition and resorption.

c. Reduction of the periodontal space in definite areas. Compression of the periodontal tissue and its blood vessels leads to hyaline degeneration of the connective tissue and in some instances to a complete obliteration of the periodontal membrane.

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[*Illustrations follow*]

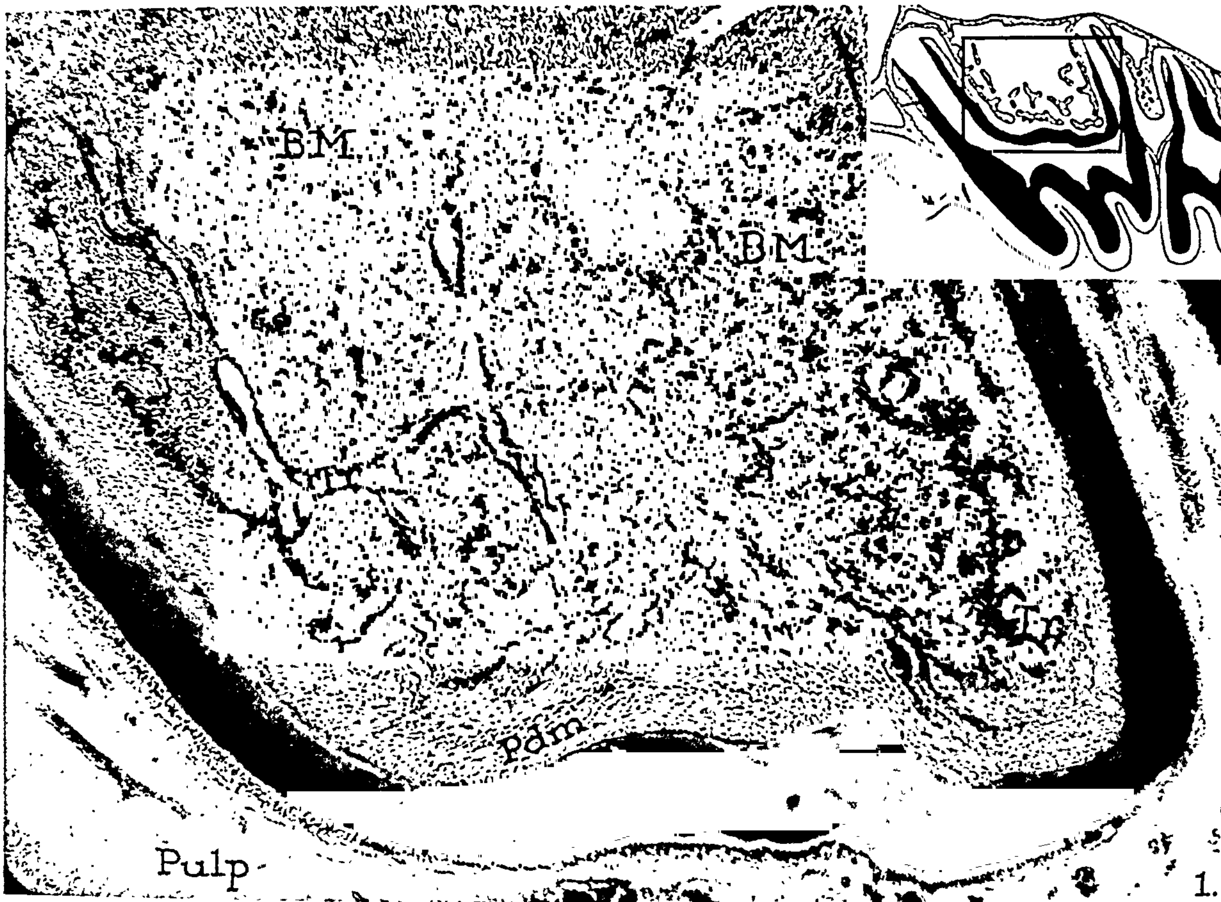
DESCRIPTION OF PLATES

PLATE 137

Photomicrographs of a mesiodistal section of the interradicular septum of a first upper molar. This area is indicated in the insert in Figure 1. × 58.

FIG. 1. Normal control rat 2901, 49 days old. Section shows thin, calcified bone trabeculae, Tr., the large marrow spaces, BM, and the even width of the periodontal membrane (Pdm.).

FIG. 2. Rat 1202, 52 days old. This animal was weaned at 21 days and then placed on the rachitogenic diet for 31 days. Section shows increased thickness of predentin (PD), cementoid (CD), and osteoid (Od.), narrowing of the marrow spaces (BM), and almost complete reduction of the periodontal membrane (Pdm.).



Weinmann and Schour

Effect of Rachitogenic Diet on Alveolar Bone

PLATE 138

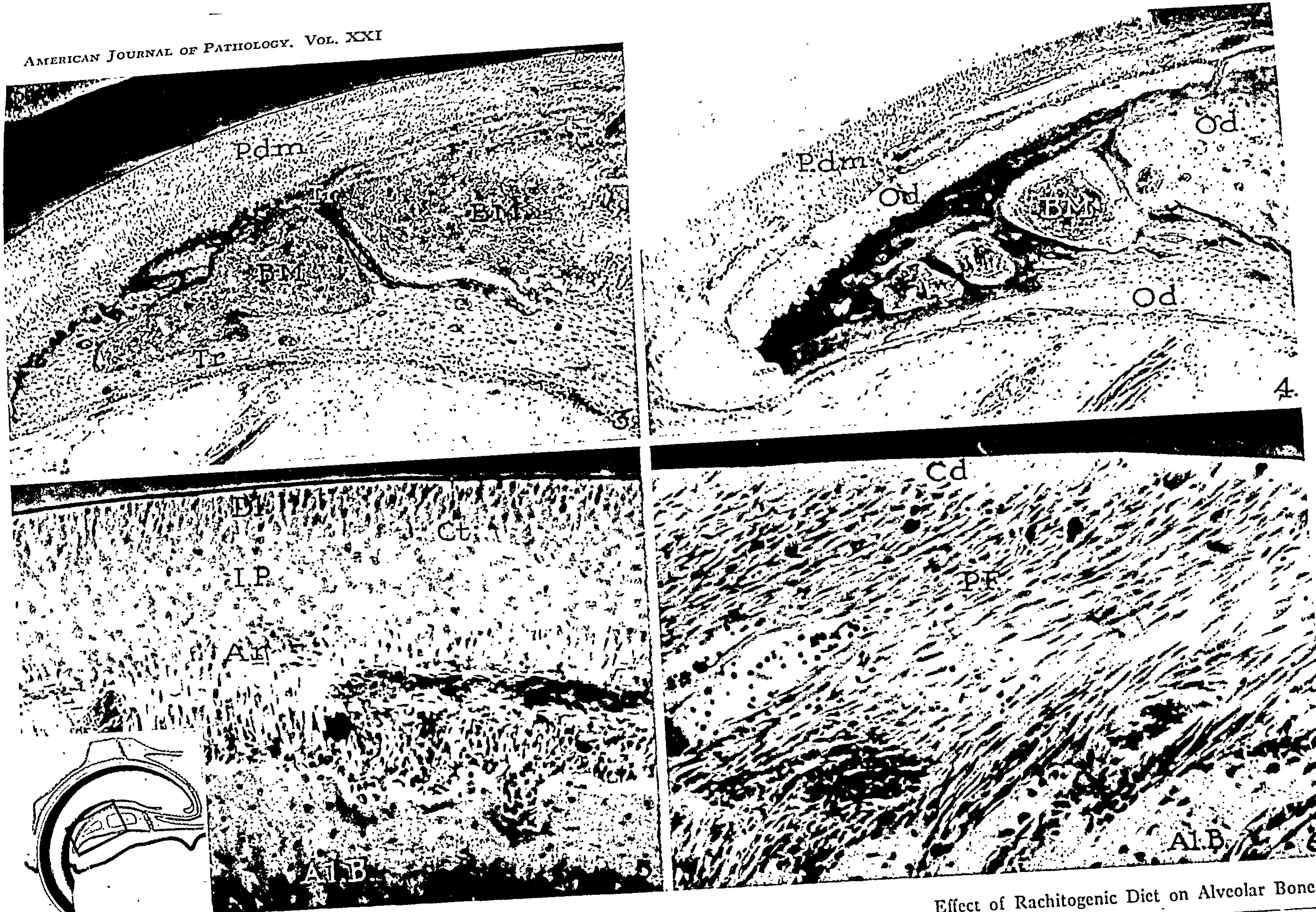
Photomicrographs of a midsagittal section of the lingual alveolar bone of the upper incisor. This area is indicated in the insert in Figure 5.

FIG. 3. Normal control rat 2901, 49 days old. The homogeneous calcification of the thin bone trabeculae (Tr) and large marrow spaces (BM) are to be noted. P.d.m. = periouontal membrane. × 52.

FIG. 4. Rat 1202, 52 days old, placed on the rachitogenic diet for 31 days after weaning. There is a thick layer of osteoid tissue (Od.) and the marrow spaces (BM) are reduced. × 52.

FIG. 5. Normal control rat 3601. Magnified area of periodontal membrane. Of note are the thin layer of cementum (Ct) and the division of the principal fibers into alveolar (Ar) and dental (Dl) fibers, and intermediate plexus (I.P.) Al.B. = alveolar bone. × 160.

FIG. 6. Rat 1802, 67 days old, placed on the rachitogenic diet for 46 days after weaning. Field corresponding to that of Figure 5, showing wide layer of cementoid tissue (Cd.) and the direct course of the principal fibers (P.F.) from alveolar bone (Al.B.) to cementum. × 160.



Weinmann and Schour

Effect of Rachitogenic Diet on Alveolar Bone

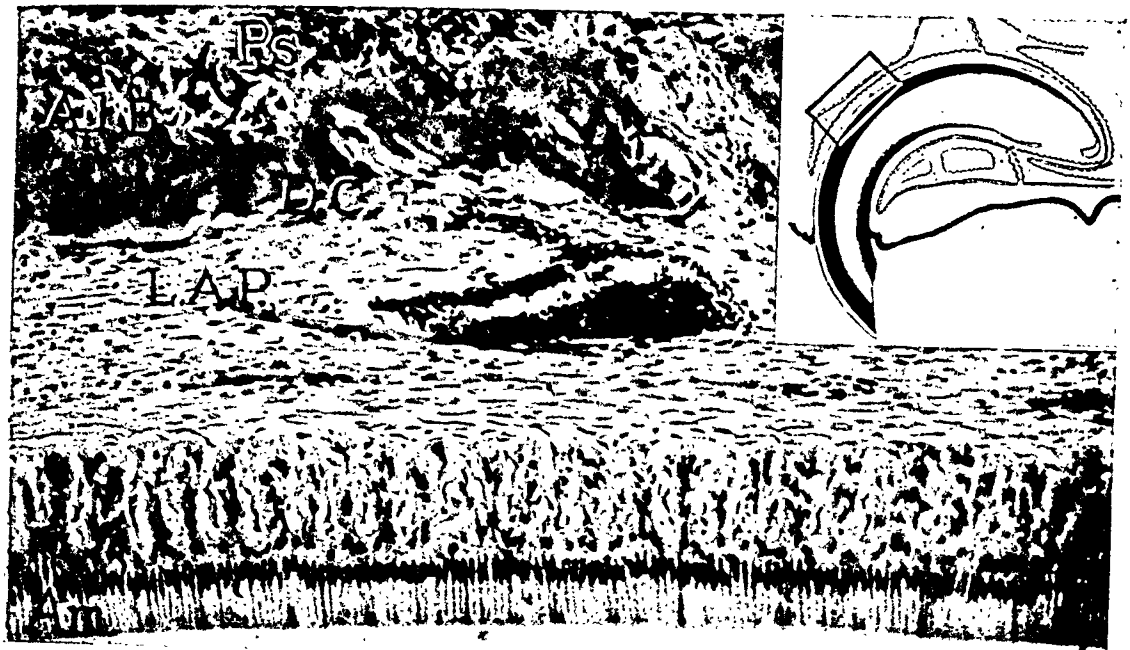
PLATE 139

Photomicrographs of decalcified midsagittal section of the labial alveolar periosteum of mid-region of upper incisor. This area is indicated in the insert in Figure 7. Am. = ameloblasts; Al.B. = alveolar bone.

FIG. 7. Normal control rat 2901, 49 days old. There is apposition of bone at the periosteal (Ps) and resorption at the dental (D.C.) surface of the alveolar bone. The width of the alveolar bone and the labial alveolar periosteum (L.A.P.) may be compared with that of Figures 8 and 9. × 172.

FIG. 8. Rat 1802, 67 days old, placed on the rachitogenic diet for 46 days after weaning. To be noted are the presence of osteoblasts on the periosteal surface (P); the absence of resorption at the dental surface of the thickened alveolar bone, which now consists of osteoid tissue (Od.); and the compression and hyaline degeneration of the labial alveolar periosteum, L.A.P. × 172.

FIG. 9. Rat 2902, 49 days old, placed on the rachitogenic diet for 28 days after weaning. Of note here are the increased thickness of the alveolar bone (Al.B.), the replacement of the labial alveolar periosteum by osteoid (Od.) and hyaline connective tissue; and the compression of the enamel organ. × 172.



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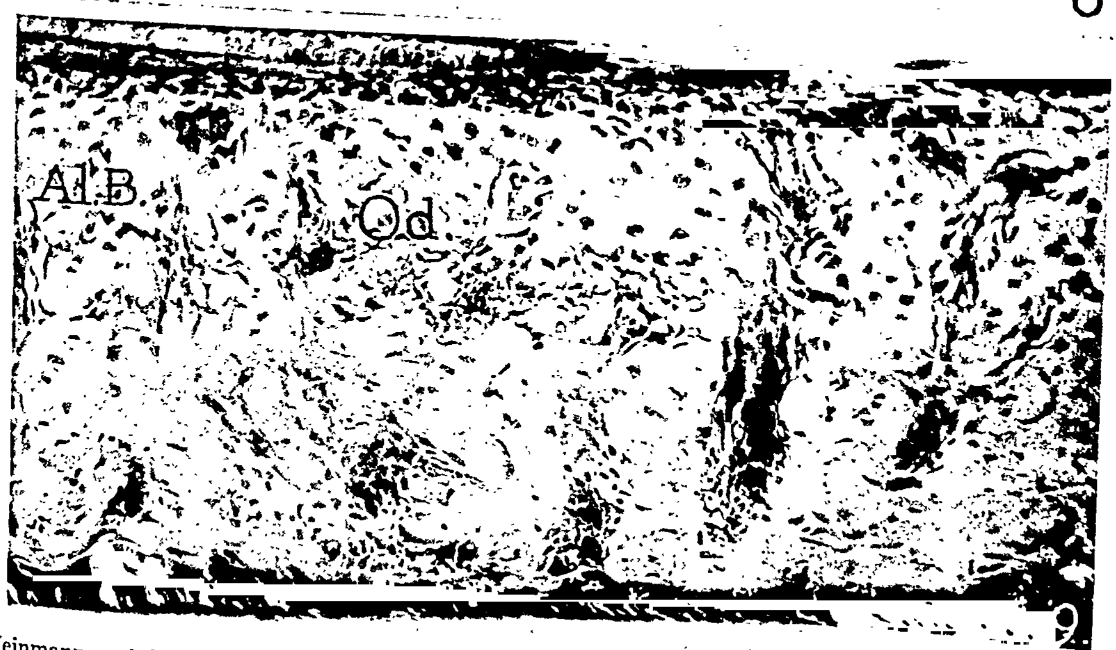

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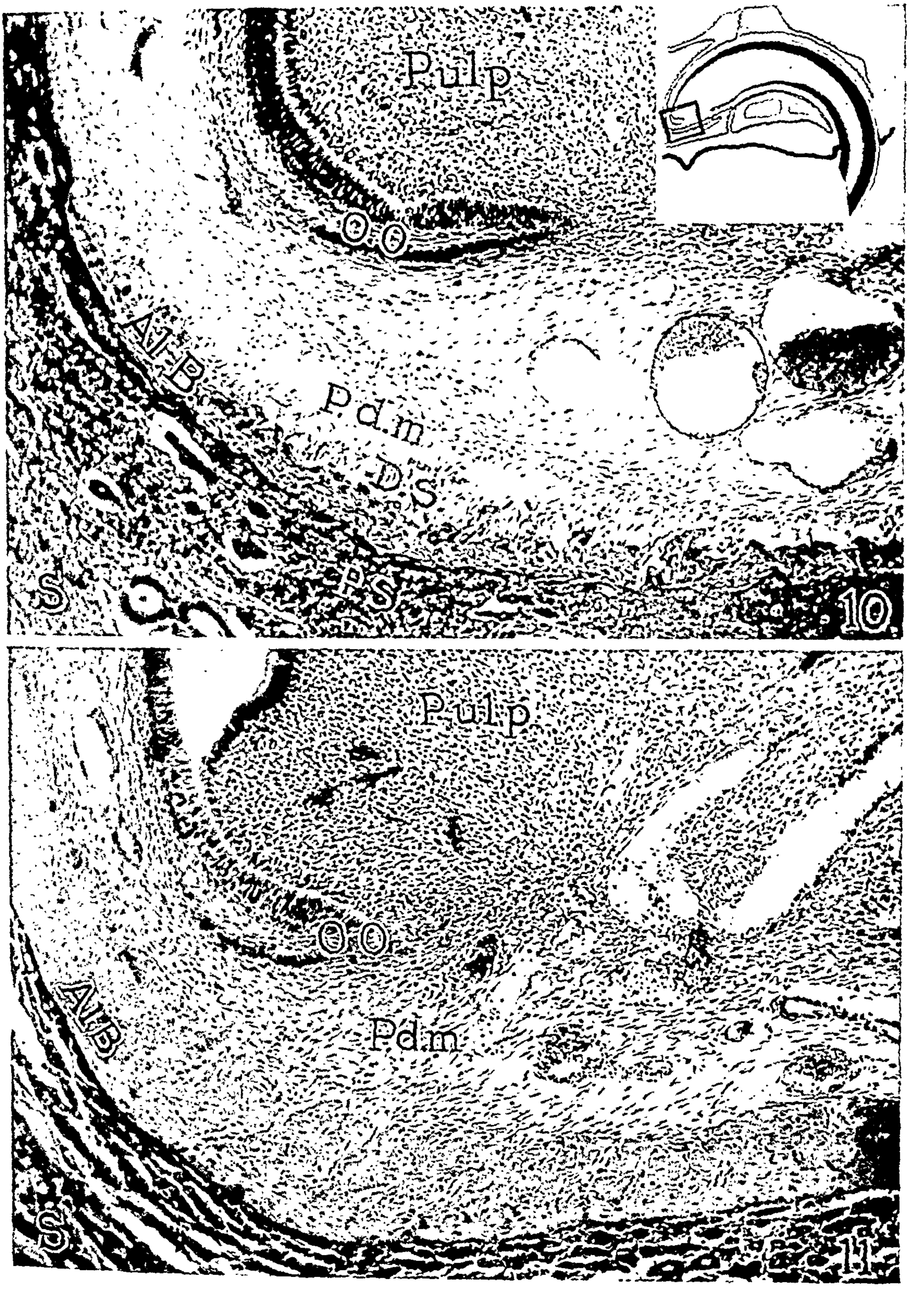
Effect of Rachitogenic Diet on Alveolar Bone

PLATE 140

Photomicrographs of decalcified midsagittal section of the labial fundic portion of the upper incisor. This area is indicated in the insert in Figure 10. Al.B. = fundic alveolar bone; O.O. = odontogenic organ; P.d.m. = periodontal membrane; S = mucous gland. ×120.

FIG. 10. Normal control rat 102, 53 days old. Apposition of bone at the periosteal surface (P.S.) and resorption at the dental surface (D.S.) of the alveolar plate.

FIG. 11. Rat 403, 53 days old, placed on the rachitogenic diet for 32 days after weaning. Reduction of the periodontal membrane (P.d.m.) in width; lack of calcification and increase of the bony plate (Al.B) in thickness; and partial replacement of the periodontal membrane by hyaline tissue are the significant features.



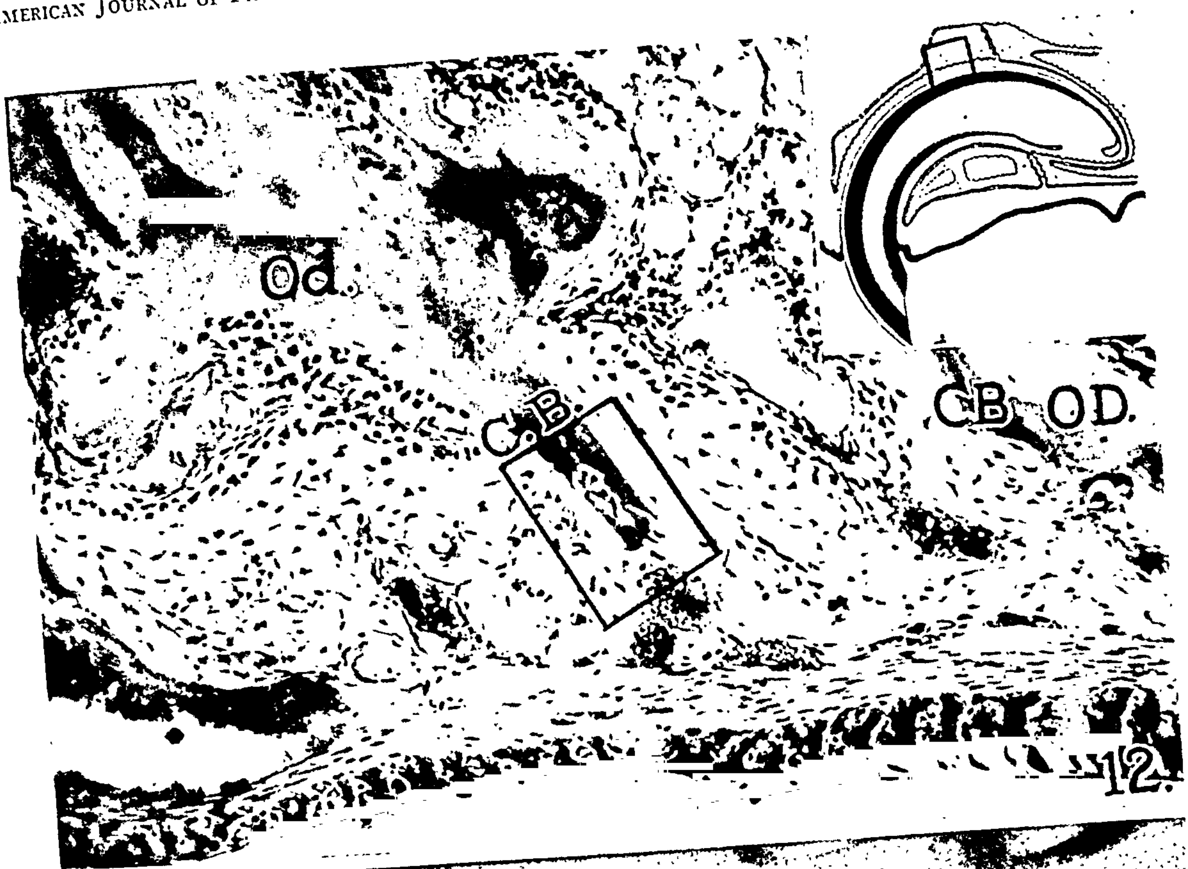
Weinmann and Schour

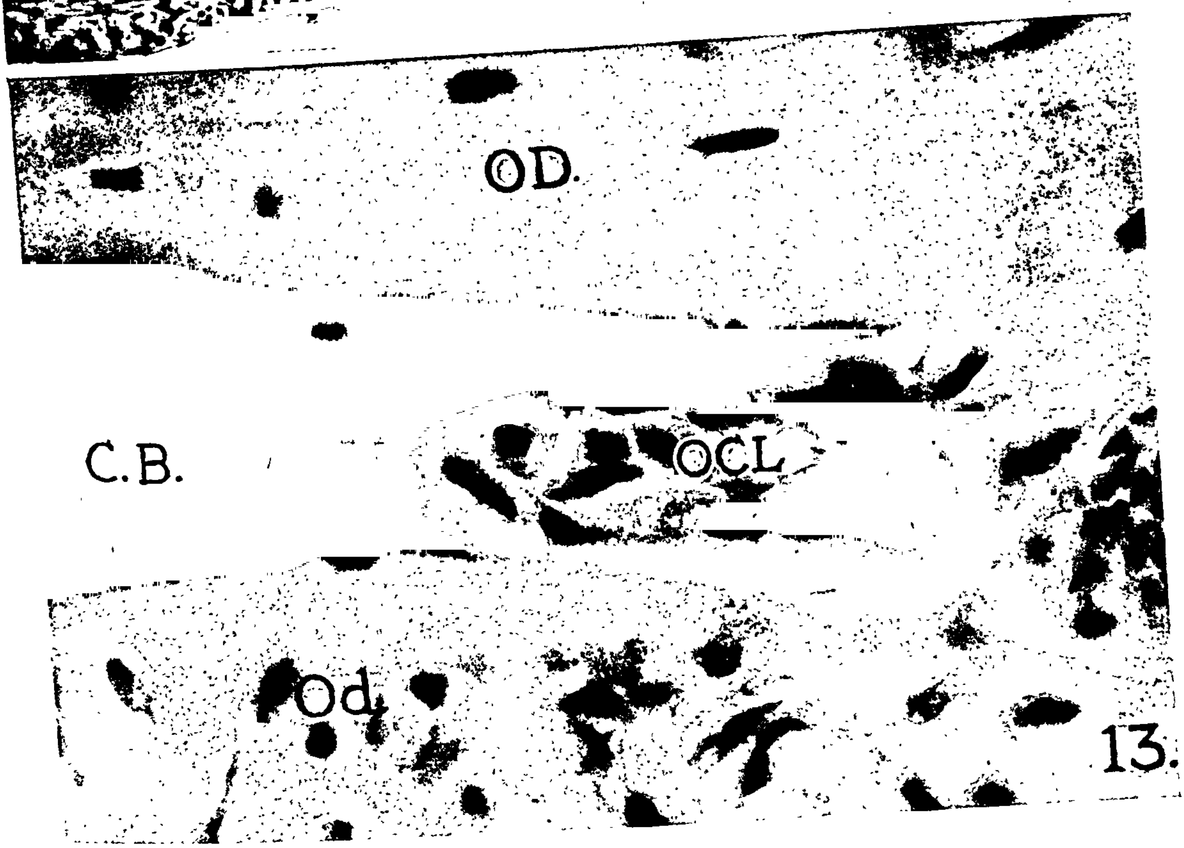
Effect of Rachitogenic Diet on Alveolar Bone

Plate 141

Fig. 12. Photomicrograph of midsagittal section of the labial alveolar bone of upper incisor of rat 2902, 49 days of age, which was weaned when 21 days old and fed the rachitogenic diet for 28 days. This area is indicated in the insert. Core of trabeculae consists of calcified bone (CB) and peripheral layers of osteoid tissue (Od.). × 150.

Fig. 13. Higher magnification of the area indicated in Figure 12, showing osteoclastic (OCL) resorption of the calcified core (CB) of the bone trabecula and resistance of the osteoid tissue (Od.) to resorption. × 800.





Weinmann and Schour

Effect of Rachitogenic Diet on Alveolar Bone

EXPERIMENTAL STUDIES IN CALCIFICATION

III. The Effect of Parathyroid Hormone on the Alveolar Bone and Teeth of the Normal and Rachitic Rat *

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In a preceding report [1] we have shown that experimental rickets is characterized by a lack of calcification of the bone matrix. The uncalcified or osteoid tissue proved to be almost immune to osteoclastic resorption. The present study was undertaken to observe the reaction of the osteoid tissue of rachitic animals in respect to the osteosclerosis and osteoclasis of hyperparathyroidism and the response of the enamel and dentin under these conditions.

Review of the Literature

Effect of Parathyroid Hormone on the Normal Bone. Bodansky, Blair, and Jaffe [2] and Jaffe and Bodansky [3] were the first to produce experimental osteitis fibrosa in guinea-pigs and dogs by injecting large doses of parathyroid hormone. They observed extensive resorption of both cortical and spongy bone and fibrous transformation of bone marrow. In growing rats, injection of parathyroid hormone resulted in osteoporosis according to Waltner [4] and Johnson,[5] and in osteosclerosis according to Bauer, Aub, and Albright.[6] Selye,[7] Shelling, Asher, and Jackson [8] and Shelling [9] explained these apparently contradictory results on the basis of the differences in the dosage of the injected hormone. Small doses produced osteosclerosis; large doses, osteoporosis. Selye suggested that the stimulating effect of the hormone on the formation of osteoblasts may be responsible for the so-called "parathyroid hormone immunity." Burrows [10] regarded the different reactions of the bone as different stages of a continuous process. McLean and Bloom,[11] by injecting extremely large doses of parathyroid hormone, showed that osteitis fibrosa appears to be a reaction to the death or injury of the cellular elements of bone.

Effect of Parathyroid Hormone on the Normal Dentition. Schour, Tweedy, and McJunkin [12] described the effect of single and multiple injections of parathyroid hormone on the incisor of the white rat. They found hypoplasia of the enamel, osteoclasis of the alveolar bone, and fibrous replacement of bone marrow. The dentin showed a primary hypocalcified layer as the immediate effect of the first injection and a

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secondary hypercalcified layer that varied with the number and dosage of the hormonal treatments. The enamel and dentin showed no resorption. Schour and Ham [13] found that the poorly calcified layer of dentin developed while the serum calcium level was rising, and that the well calcified layer developed during the period when the serum calcium level was falling.

Effect of Parathyroid Hormone on the Rachitic Bone. Shelling, Asher, and Jackson[8] found that parathyroid hormone in doses of 10 units daily failed to protect the animals against rickets. They found a moderate amount of submetaphyseal sclerosis but the newly formed bone consisted of poorly calcified trabeculae. McLean and McCoy [14] found that doses of parathyroid hormone sufficiently large to cause osteoporosis led to a dense deposit of calcium and phosphate in rachitic cartilage.

The effect of parathyroid hormone on dentition in rachitis has not been reported as far as we were able to ascertain.

Material and Method

This study is based on the histologic sections of the upper jaws of two groups of white rats.* Group I consisted of 23 control animals that were fed a normal basal diet (Table I). Group II consisted of 38 animals that were placed on the rachitogenic diet of Steenbock and Black [15] (Table II). At ages of 48 to 52 days the animals of both groups were injected with parathyroid hormone. The doses varied from 1 to 24 in number and from 50 to 780 Hanson units in quantity. The animals were killed from 1 to 132 hours after the last injection and their ages at death ranged from 48 to 73 days. An additional control group consisted of 16 normal and 17 rachitic rats of similar age.

The heads of the experimental and control rats were removed and fixed in 10 per cent formalin immediately after sacrifice. The jaws were dissected and the upper incisors and molars were prepared for decalcified histologic sections. The incisors were cut in serial longitudinal sections and the molars in serial mesiodistal sections. The stains used were hematoxylin and eosin.

Findings

I. Alveolar Bone

The effect of the injection of parathyroid hormone on bone depends primarily upon the number of units in each single dose and secondarily upon the total number of units when multiple injections are used. The

* The animals used in this series of studies were kindly made available by Drs. F. C. McLean and W. Bloom.

difference in the effects of an injection of 50 Hanson units and 100 or more Hanson units is qualitative rather than quantitative.

A. *The Effect of Small Doses (50 Hanson Units)*

Animals Placed on Normal Basal Diet. The alveolar process of an animal injected with three daily doses of 50 Hanson units each showed the signs of increased osteoclastic and osteoblastic activity. Some of the osteoclasts had disappeared and the Howship's lacunae in some places were covered by newly formed bone. With the increase in the number of injections, a major portion of the large marrow space of the normal alveolar process (Fig. 1) was gradually replaced by a network of well calcified bone trabeculae (Fig. 2). These were densely bordered by apparently very active osteoblasts. The newly formed bone was more intensively stained than were the pre-experimental bone trabeculae. The marrow lined by the newly formed bone trabeculae was fibrous. Only small areas of red marrow were left.

Rachitic Animals. The reaction of rachitic bone to the injection of small doses (Table II) was similar to that seen in the normal animals but was less intense. A significant change could be noticed after nine daily injections of 50 Hanson units each. The osteogenic activity of the endosteum and periosteum appeared markedly stimulated. After the 18th day of the experiment the width of the osteoid border on the basal plate and along the trabeculae of the spongy bone had increased. The large bone marrow spaces of the rachitic animal (Fig. 3) appeared to be almost obliterated in the animal treated with parathyroid extract (Fig. 4).

The changes in the alveolar process were similar in the regions of the upper incisor and of the molar.

B. *The Effect of Large Doses (100 or More Hanson Units)*

Animals Placed on Normal Basal Diet. The number of osteoclasts increased following a single injection of 200 units of parathyroid hormone (Figs. 5 and 6). The osteoclasts were found along the trabeculae in the small as well as the large marrow spaces and along the periodontal surface of the alveolar bone. They were missing at the crest of the septa. In the areas where osteoclasts were seen, the bone was scalloped in outline (Howship's lacunae) but did not differ in staining from bone which was covered by resting and inactive periosteum or endosteum.

The number of osteoclasts and Howship's lacunae increased with an increasing total dosage of parathyroid hormone, when the single dose was 100 units or more. In the molar region, the destructive process

TABLE I

Data on 23 Rats That Were Fed a Normal Diet and Were Given Injections of Parathyroid Hormone

Rat number	Age at death	Duration of experiment	Dosage in units (Hanson)	Number of injections	Total units	Hours between last injection and death	Resorption of alv. process		Hyper-osteogenesis
							Molar region	Incis. region	
3102	52 days	3 days	50	3	150		N	N	+
3103	55 days	6 days	50	6	300		N	N	+
3104	58 days	9 days	50	9	450		N	N	++
3105	61 days	12 days	50	12	600		N	N	++
3106	64 days	15 days	50	15	750		N	N	++
3107	67 days	18 days	50	18	900		N	N	++
3108	70 days	21 days	50	21	1050		N	N	++
3109	73 days	24 days	50	24	1200		N	N	++
3002	49 days	9 hrs.	200*	5	1100	5	N	N	N
3003	49 days	12 hrs.	200*	5	1100	8	N	N	N
3602	53 days	1 day	100	2	200	24	+	+	N
2302	53 days	1 day	100	2	200	24	+	+	N
103	50 days	1 day	100	2	300	0	+	N	N
2303	54 days	2 days	100	4	400	24	+	+	N
3603	54 days	2 days	100	4	400	24	+	+	N
104	51 days	3 days	100†	4	500	0	++	N	N
2304	55 days	3 days	100	6	600	24	++	±	N
3604	55 days	3 days	100	6	600	24	++	+	N
105	52 days	4 days	100†	6	700	0	+++	+	N
3605	56 days	4 days	100	8	800	24	+++	+	N
106	53 days	5 days	100†	8	900	0	++++	+	N
3606	57 days	5 days	100	10	1000		+++	++	N
3005	53 days	5 days	200*	5	1100	82	N	N	N

N = No recognizable deviation from normal.
* First injection was 300 units.
† First injection was 200 units.

TABLE II

Data on 38 Rats That Were Fed a Rachitogenic Diet and Given One or Multiple Injections of Parathyroid Hormone

Rat number	Weight at death	Sex	Age at death	Duration of experiment	Dosage units	Times injected	Total units	Hours between last injection and death	Histologic findings: Resorption of alveolar process, Molar region	Histologic findings: Resorption of alveolar process, Incis. region	Histologic findings: Hyper-osteogenesis
	(gm.)		(days)								
1803	85		52	3 days	50	3	150	24	N	N	N
1804	89	M	55	6 days	50	6	300	24	N	N	N
1805	84	F	58	9 days	50	9	450	24	N	N	+
1806	111	M	61	12 days	50	12	600	24	N	N	+
1807		M	64	15 days	50	15	750	24	N	N	+
1808	73	M	67	18 days	50	18	900	24	N	N	+
1502	58	M	48	1 hr.	200	1	200	1	N	N	N
1303		M	49	1 hr.	480	1	480	1	N	N	N
1503	57	M	48	2 hrs.	200	1	200	2	N	N	N
1304		M	49	2 hrs.	780	1	780	2	N	N	N
1504	62	F	48	3 hrs.	200	1	200	3	N	N	N
1305		F	49	4 hrs.	600	1	600	4	N	N	N
1506		M	48	8 hrs.	200	1	200	8	N	N	N
1306		F	49	8 hrs.	740	1	740	8	N	N	N
3505		M	50	1 day	100	1	100	24	N	N	N
1203	65	F	49	1 day	160	1	160	24	N	N	N
304	66	F	50	1 day	200	1	200		N	N	N
1604		M	50	1 day	200	1	200	24	N	N	N
1205	69	M	50	1½ days	180	1	180	36	+	N	N
3506		F	51	2 days	100	2	200	24	+	N	N
1605			51	2 days	200	2	400	48	+	N	N
25			50	2 days	100	5	500	6	++	N	N
27			50	2 days	100	5	500	6	+	N	N
1206	72	F	51	2½ days	200	1	200	60	+	N	N
3507		F	52	3 days	100	3	300	24	+	N	N
703	74	M	52	3 days	100	4	400	0	N	N	N
306	47	F	52	3 days	200	3	600		++	±	N
1606	95	M	52	3 days	200	3	600	24	++	N	N
1207	76	M	52	4 days	180	1	180	84	N	N	N
3508			53	4 days	100	4	400	24	+	N	N
307	55	F	53	4 days	200	4	800		++	N	N
1607		M	54	4 days	200	4	800	4	++	+	N
1208	72	M	53	5 days	190	1	190	108	N	N	N
1209	82	F	54	6 days	200	1	200	132	N	N	N
704	74		55	6 days	100	7	700	24	++	N	N
305	61	F	57	8 days	200	2	400		+	N	N
705	90		59	9 days	100	9	900	24	++	++	N
706	88		62	11 days	100	11	1100	24	++	+	N

N = No recognizable deviation from normal.

affected, first, the basal portion (Fig. 6) of the alveolar process and, later, the alveolar crest (Fig. 7). Daily injections of 200 units led to a complete disappearance of the alveolar bone after 5 days (Fig. 8). The alveolar bone was then replaced by connective tissue containing numerous fibroblasts, osteoclasts, and capillaries. The osteoclasts and fibroblasts were grouped in strands. A large part of the red bone marrow and of the periodontal membrane appeared unchanged even in the most advanced stages of hyperparathyroidism. The principal fibers of the periodontal membrane could be traced into the fibrous tissue which had replaced the bone trabeculae (Fig. 8).

The increase of osteoclasts was not noticed until 24 hours following a single injection of large dosage. Even the injection of massive doses of 1100 units did not cause an increase of osteoclasts within 12 hours. The reaction disappeared on the fourth day following a single injection.

Rachitic Animals. The reaction of rachitic alveolar bone to the injection of large doses of parathyroid hormone differed from that of normal bone only in degree (Table II). Following the injection of 200 or more Hanson units of parathyroid hormone, large numbers of osteoclasts were present in the alveolar process of the molar region. Twenty-four hours after the injection, fibrous tissue replaced part of the bone trabeculae. Most of the osteoclasts were found adjacent to the calcified part of the trabeculae (Fig. 10). A comparison of the alveolar bone of the rachitic and of the normal rat after the injection of the same doses of parathyroid hormone revealed that osteoclasis was far more advanced in the calcified portion than in osteoid tissue (compare Figs. 8 and 10). The osteoid portion of the trabeculae resisted resorption much longer than did the calcified portion.

The reaction of the alveolar bone was less intense in the upper incisor area than in the molar region. This was true for the normal (Figs. 11 and 12) as well as for the rachitic animal. In animals placed on a basal diet and given injections of parathyroid hormone, the bony plates of the alveolar bone of the incisors were reduced in thickness. In no site was there as complete a replacement by fibrous tissue as in the alveolar process of the molars, not even in those areas where internal resorption is normally found and where the bony plate is exceedingly thin. In rachitic animals the alveolar bone of this area showed only slight changes which could be attributed to the injection of parathyroid hormone.

II. DENTAL TISSUES

Upper Molars. The enamel and dentin of the upper molars showed no resorption and the incidence of root resorption was not significantly different from that found in the control animals. The recently formed dentin showed a hypercalcified stripe.

Upper Incisor. Injection of 50 Hanson units of parathyroid hormone, even repeated as many as 24 times, did not cause significant changes in the tissues of the incisors. Multiple injections, each of 100 or more Hanson units, in the control rats placed on a basal diet resulted in a stratification of the dentin. The dentin calcifying on the first day following the injection remained hypocalcified and appeared as a pink stripe which had the same width as the predentin. The later dentin was hypercalcified and stained intensively with hematoxylin. The striation was more pronounced in the middle and incisal thirds of the labial dentin. These observations are in complete agreement with the findings of Schour and his collaborators.[12, 13]

The changes in the dentin of rachitic rats following injections of parathyroid hormone were of questionable significance. In the incisal third the layer of dentin next to the predentin stained deeply with hematoxylin, indicating some hypercalcification. The uncalcified basal end (basal predentin) of the incisor seemed to be shorter. On the other hand, the width of the predentin and the frequency of the intermediate dentin were the same as in the control rachitic rats.

The injections of parathyroid hormone had no significant effect on the enamel organ or the enamel.

Discussion

The Paradoxic Effects of Small and Large Doses of Parathyroid Hormone

Our observations on the alveolar bone are in agreement with the findings in long bones reported by Shelling, Asher, and Jackson [8] and by Selye[7] who found that massive doses produce osteitis fibrosa, but small doses cause osteoblastic activity and an increase in the number of bone trabeculae. Shelling and his collaborators refrained from explaining this paradoxic reaction. Burrows [10] distinguished three phases in the effect of parathyroid hormone. The first phase is characterized by the resorption of bone. In the second phase osteoblasts reappear and excessive bone deposition occurs. In the third stage there is a tendency of the bone to return to a normal state. Burrows, in agreement with Selye's explanation, regards the sequence of changes as "due to the development of an antibody-like substance in the blood which acts as a check on decalcification and in its stead introduces hypercalcification."

As far as our material permitted, we were able to differentiate two stages, but only after injections of small doses of parathyroid hormone. The osteoblastic activity followed a short period of increased osteoclasis. It led to osteosclerosis. A return to normalcy could not be observed in our animals. We agree with the explanation offered by Selye [7]

and Burrows [10] that injections of parathyroid hormone of bovine origin might cause the formation of an antibody in the rat. After a short interval of osteoclasis these antibodies could be produced in excess and would neutralize not only the injected hormone but also some of the parathyroid hormone produced by the glands of the animal. However, with the injection of massive doses the production of antibodies is not sufficient to overcome the surplus of hormone. Small doses would in this way lead in the end to hypoparathyroidism and large doses would produce the symptoms of hyperparathyroidism.

Experimental proof of this hypothesis is not yet available, but the clinical observations tend to support it. Aub, Albright, Bauer, and Rossmeisl [16] found that parathyroid extract gradually loses its therapeutic effect in tetany after long-continued administration. They assumed that the patient acquired active immunity to the injected hormone. Furthermore, Kendall, Walsh, and Howard [17] observed that idiopathic hypoparathyroidism causes overproduction in bone as shown by its increased density in roentgenograms.

Osteoclasis in hyperparathyroidism should be regarded as a pathologic exaggeration of the physiologic process of bone resorption which is an integral factor in bone development and bone growth. Resorptive processes are entirely lacking in the development and growth of the tooth, with the exception of the shedding of deciduous teeth. This might explain the freedom of enamel, dentin, and cementum from resorption in hyperparathyroidism.

Reaction of Rachitic Bone to Parathyroid Hormone

The reactions of rachitic and of normal bone to injection of parathyroid hormone were essentially the same. In both instances new bone formation was stimulated by small doses and resorption of bone was caused by large doses. A comparison of the two groups of experiments, however, showed a definite difference in the reaction following injection of large doses. In all instances the resorption of the rachitic bone was less advanced than was the resorption of bone of the normally fed rats under otherwise similar conditions. The explanation for this striking difference is found in the fact that bone which is uncalcified (osteoid tissue) is more resistant to resorption than calcified bone. That the degree of calcification might play a rôle in the susceptibility to resorption was suggested by Gottlieb [18] and substantiated by Orbán and Weinmann [19] and Kronfeld.[20] Strong support of the theory is seen in the findings reported in the preceding communication [1] by which it could be demonstrated that osteoid tissue is relatively immune to resorption.

Differences in Reaction of the Alveolar Bone of Molar Region and of Incisor Region

Differences in reaction of various areas of bone to the parathyroid hormone were observed by Jaffe [21] who stated that "the sites of most active bone formation are the sites most susceptible to resorption." It is likely that the greater resorptive activity in the alveolar process of the molar area as compared with that in the region of the incisor is a result of the greater growth activity of the former.

Summary and Conclusions

The effect of single and multiple injections of parathyroid hormone on the upper jaws of the white rat was studied histologically in 23 animals fed a normal diet and in 38 animals placed on a rachitogenic diet after weaning. Sixteen normal and 17 rachitic rats from 42 to 77 days of age were used as controls. The histologic findings were:

1. Injections of 50 Hanson units of parathyroid hormone stimulated osteoblastic activity and new formation of alveolar bone.
2. Injections of 100 or more Hanson units of parathyroid hormone resulted in progressive osteoclasis of the bone and replacement of the bone by fibrous tissue.
3. The reaction of the bone to parathyroid hormone was essentially the same in normal and rachitic rats, except for the fact that the osteoid tissue in the latter was more resistant to resorption.
4. Following injections of large doses of parathyroid hormone, the alveolar bone of the molar areas showed more severe changes than that of the incisor region.
5. Injections of parathyroid hormone do not cause resorption of the hard dental structures (enamel, dentin, and cementum).

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[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 142

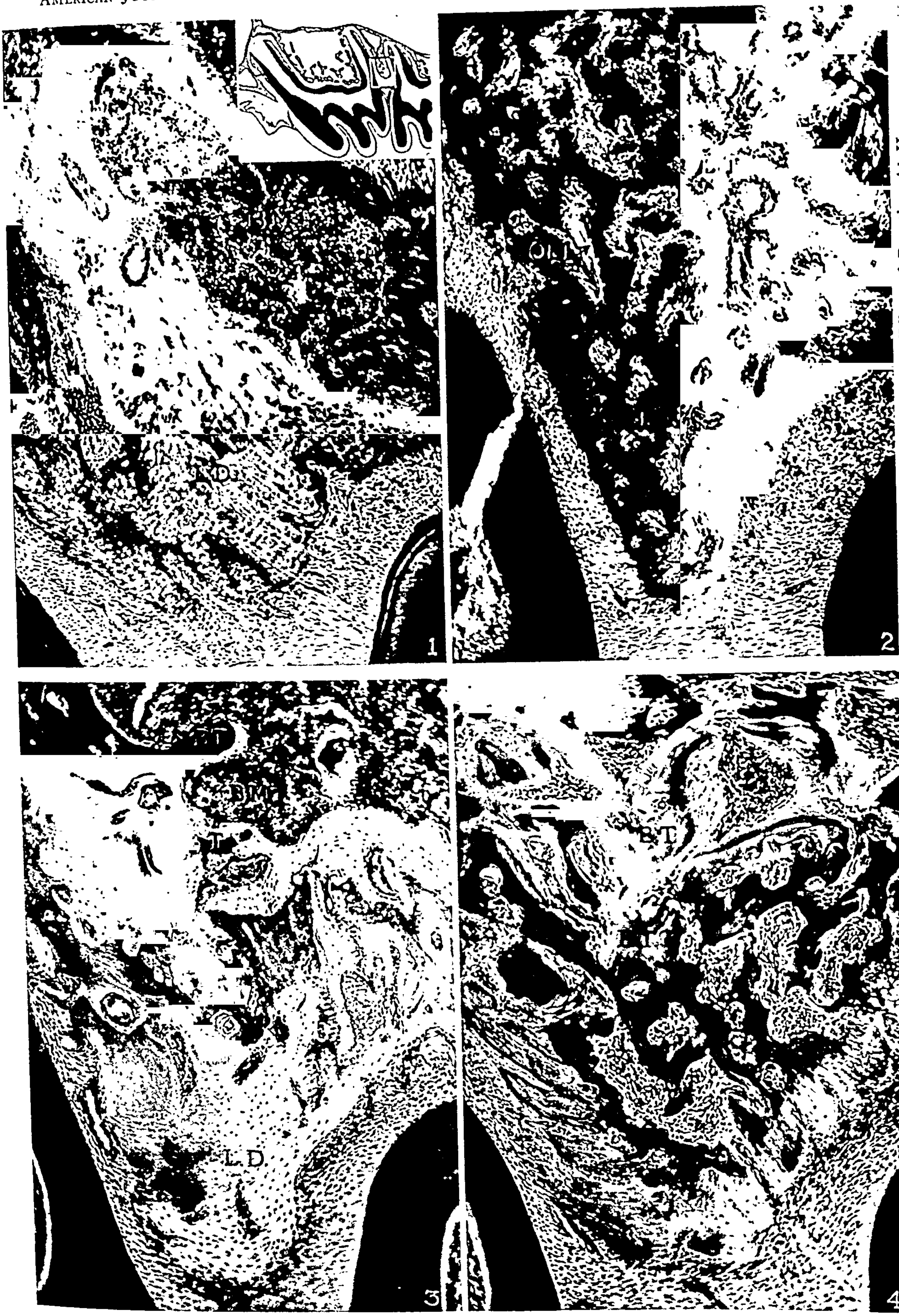
Photomicrographs of decalcified mesiodistal sections of the interdental septum between the first and second upper molars showing the effect of small doses of parathyroid hormone on the normal and rachitic alveolar bone. Area is indicated in the insert in Figure 1. B.M. = bone marrow; B.T. = bone trabeculae. × 70.

FIG. 1. Rat 1801, 67 days old. Normal control. The lamina dura (L.D.) is thick and well calcified and the bone marrow spaces (B.M.) are large.

FIG. 2. Rat 3109, 73 days old. Normal diet. Twenty-four daily injections of parathyroid hormone, each 50 Hanson units, were administered. A network of calcified bone trabeculae (B.T.) replaces the pre-experimental bone and fills a large part of the bone marrow spaces. The bone trabeculae are bordered by osteoblasts (Obl.). At a few places osteoclasts can be seen.

FIG. 3. Rat 1802, 67 days old. Rachitogenic diet. The width of the lamina dura (L.D.) and of the bone trabeculae (B.T.) is increased. The core of the bone trabeculae is calcified and covered by thick layers of osteoid tissue. The bone marrow spaces (B.M.) are narrow.

FIG. 4. Rat 1808, 67 days. Rachitogenic diet for 46 days after weaning. Eighteen daily injections of parathyroid hormone, each of 50 Hanson units, were administered. The thick lamina dura is replaced by a network of bone trabeculae which are covered by a layer of osteoid tissue and by osteoblasts, indicating progressing bone apposition. The large bone marrow spaces are partly filled by newly formed bone trabeculae (B.T.).



Weinmann and Schour

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PLATE 143

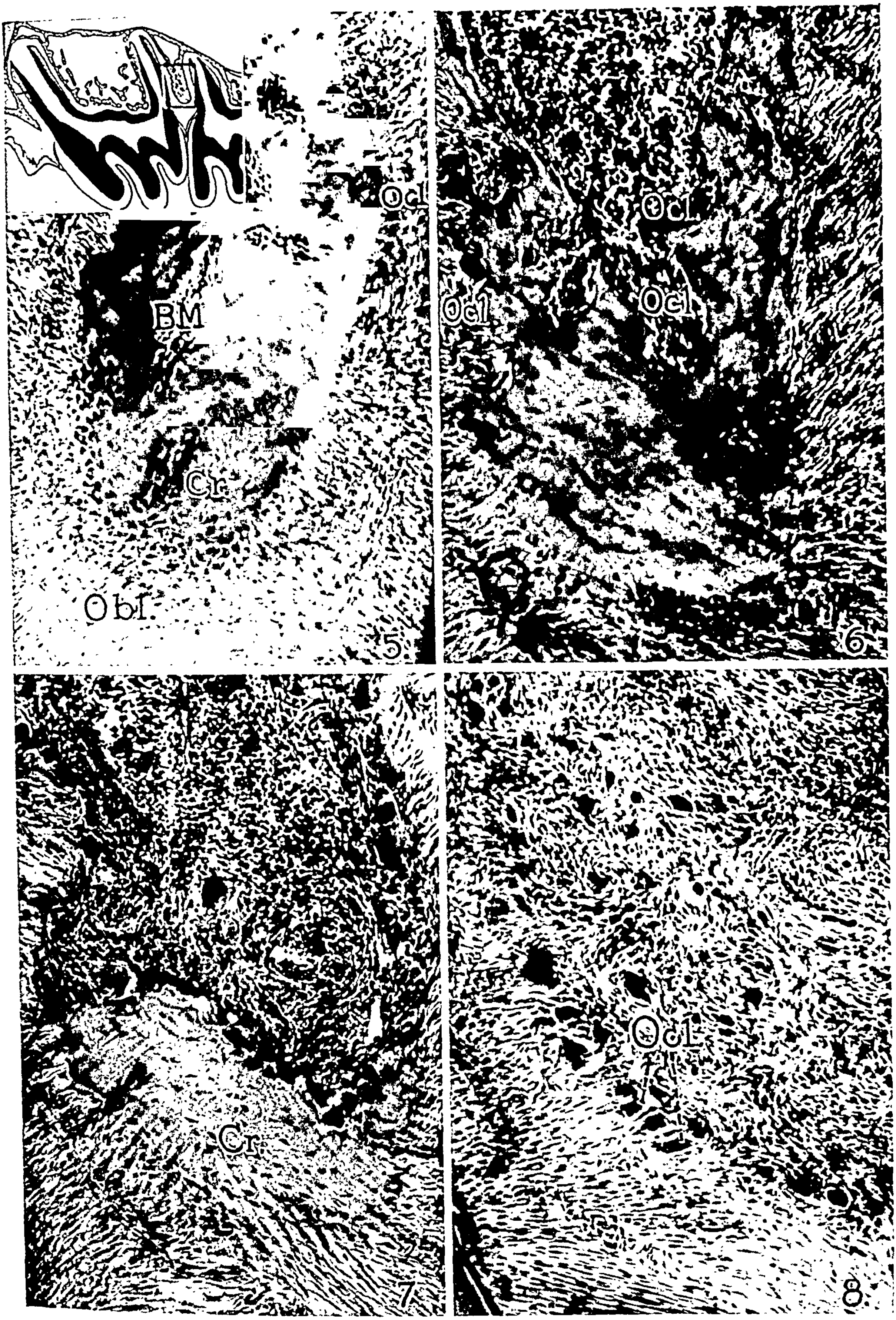
Photomicrographs of decalcified mesiodistal sections of the interdental alveolar septum between first and second upper molars showing the effect of large doses of parathyroid hormone on the alveolar bone of rats kept on a normal basal diet. Area is indicated in the insert in Figure 5. × 135.

FIG. 5. Rat 2901, 49 days old. Normal control. The bone trabeculae are well calcified, the largest forming the crest (Cr.) covered by osteoblasts (Obl.); in the lateral parts few osteoclasts (Ocl.) are seen. Bone marrow spaces (B.M.) are small.

FIG. 6. Rat 103, 50 days old. Two injections of a total of 200 Hanson units of parathyroid hormone were administered. There is an increased number of osteoclasts (Ocl.) at the periodontal and endosteal surfaces.

FIG. 7. Rat 104, 51 days old. Four injections of a total of 500 Hanson units of parathyroid hormone were administered within 3 days. Progressive osteoclasis may be noted. The alveolar bone is resorbed except for a small cap of bone at the crest (Cr.).

FIG. 8. Rat 106, 53 days old. Eight injections of a total of 900 Hanson units of parathyroid hormone were administered within 5 days. The alveolar bone is resorbed by osteoclasts (Ocl.) and replaced by fibrous tissue.



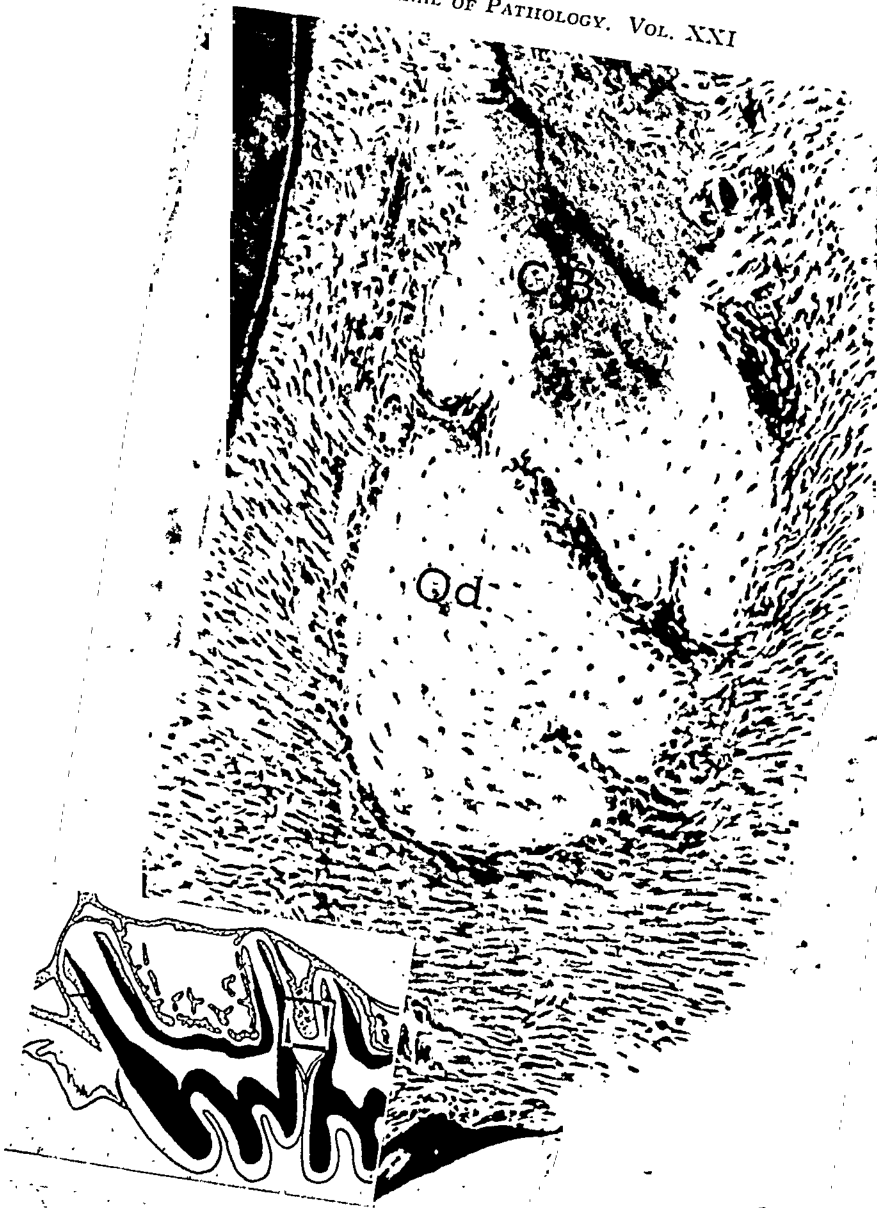
Weinmann and Schour Effect of Parathyroid Hormone

Plate 144

Photomicrographs of decalcified mesiodistal sections of the interdental alveolar septum between first and second upper molars showing the effect of larger doses of parathyroid hormone on the alveolar bone of rats kept on rachitogenic diet. Area is indicated in the insert in Figure 9. × 140.

Fig. 9. Rat 1202, 54 days old. Rachitic control. The alveolar ridge is formed by osteoid tissue (Od.); the basal part is calcified (C.B.).

Fig. 10. Rat 307, 53 days old. Four injections, of parathyroid hormone, totalling 800 Hanson units, were administered. Resorption is active primarily in the calcified bone, which is replaced by fibrous tissue (F.T.) rich in giant cells, while the larger part of the osteoid tissue (Od.) is left intact.

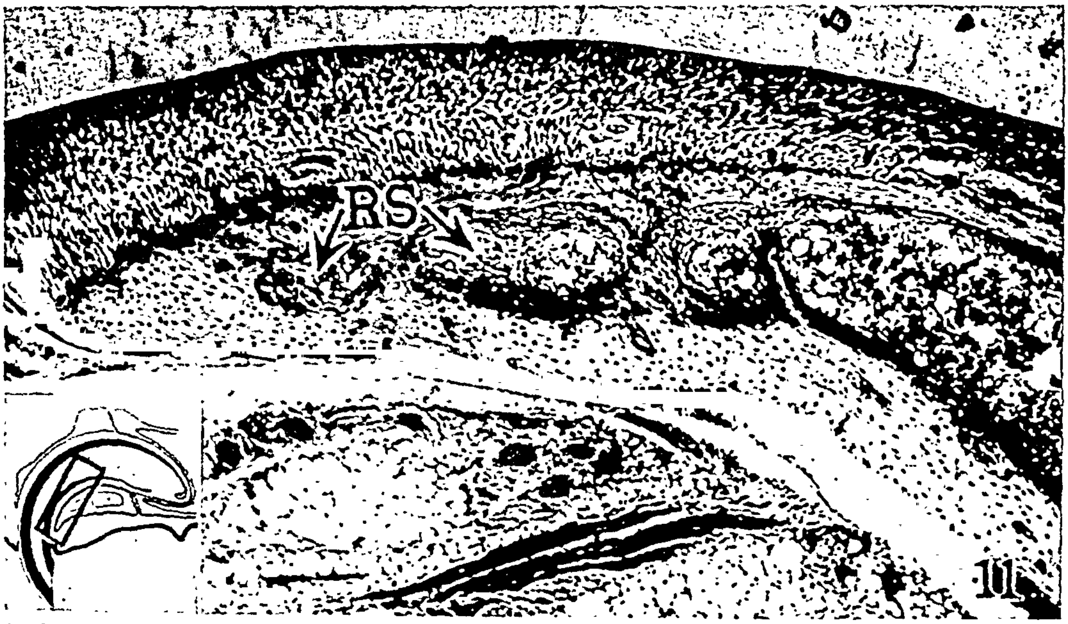


9.

10

PLATE 145

Photomicrographs of decalcified sections showing the different reactions of the lingual alveolar bone of the upper incisor (*Fig. 11*) and of the interradicular septum at the bifurcation of the first upper molar (*Fig. 12*) of rat 106, 53 days old, kept on the basal diet and administered a total dosage of 900 Hanson units of parathyroid hormone. While the alveolar bone of the molar is entirely resorbed and replaced by fibrous tissue (F.T.), the alveolar bone of the incisor shows only slight resorption (R.S.). Areas are indicated in the inserts. $\times$ 90.



Weinmann and Schour

Effect of Parathyroid Hormone

A STUDY OF THE CIRCULATION OF THE SPLEEN IN SICKLEMIA AND SICKLE CELL ANEMIA *

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Numerous reports [1-3] are available describing the histologic changes in the spleens of Negroes whose erythrocytes showed sickled forms. No reports are found attempting to correlate these changes with the bizarre shapes of the erythrocytes. Rich [1] stated that the changes in the spleen are congenital in origin. In the study of a large number of cases of sicklemia and sickle cell anemia the failure to find evidence of congenital vascular abnormalities in the spleens, and the possibility that the splenic picture described as being typical of the sickling phenomenon may be the result of the sickled cells, led to the studies reported here.

Normal Circulation of the Spleen

The circulation of the normal spleen has been a controversial subject and is dealt with in detail by Klemperer [4] who gives the current concept as follows:

> "Because of the net-like structure of the arterial terminations, as well as of the venous sinuses, the circulation is evidently open. However, the width of the stomata is variable, and is influenced by various factors. For instance, contraction of the spleen will cause a diminution of the size of the pores, whereas increased tension within the vascular channels provoked by elevation of the arterial or venous pressure will result in dilatation of the stomata within the vascular wall. . . . It is evident that if the pores in the vascular channels are wide open, the blood will escape freely into the surrounding pulp spaces, whereas if they are narrow, the blood will remain within a pathway separated from the surrounding spongy spaces of the pulp by a perforated but very narrow meshed wall."

MacNeal, Otani, and Patterson,[5] in a series of injection preparations of spleens, classified the capillaries of the spleen into three groups: (1) follicular capillaries, (2) capillaries of marginal zone, and (3) capillaries of the pulp cords. They felt that the follicular capillaries arose from the central arteries, passed through the substance of the follicle and terminated just beyond the marginal zone by numerous small openings into the red pulp, always at considerable distances from the nearest venous sinuses. The follicular capillaries were frequently united by short branches. In some instances the endings were distended, presenting an ampulla, with restriction of erythrocytes and allowing the escape of plasma through the endothelial walls. In other instances there were large openings between the endothelial cells permitting the escape of erythrocytes into the pulp cords and, from them, into the venous sinuses through the stomata of Mollier.[6] The capillaries of the marginal zone arose from branches of the follicular capillaries passing into the (red) pulp where they branched, and the small branches terminated in

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the marginal zone of the follicle in the pulp spaces as the Schweigger-Seidel capillaries. The tips of these capillaries were usually distended to thin-walled ampullae with narrow clefts leading into the pulp spaces of the marginal zone. The capillaries of the pulp cords were branches of the capillaries of the marginal zone and terminated in the pulp spaces, with or without ampullae, and in some instances emptied directly into the venous sinuses. These authors thought that the circulation of blood in the spleen was open, with the pulp spaces constituting the connections between the arterial and venous channels.

Robinson [7] likewise came to the conclusion that the circulation of the spleen was open, and that the pulp spaces were the only communications between the arteries and veins. In regard to the arterial terminations he stated:

> ". . . . Golz, a co-worker of Thoma, using a granular injection mass, showed that the arterioles passed through the ellipsoids and ended in ampullatous dilatations of the vessels. These findings were confirmed by Mall who designated the dilatation of the capillary as 'the ampulla of Thoma.' "

Robinson concluded that the ampullae of Thoma were the funnel-shaped capillary endings in the red pulp cords, and that those seeming to be dilated into ampullae were merely exaggerated pulp spaces.

Material

The material studied can be divided into two parts. The first part is composed of 150 autopsies in which sickled erythrocytes were found in routine sickling preparations as reported elsewhere.[8] Sections of spleen stained by hematoxylin and eosin following formol-alcohol fixation were available in all these cases, and also included are 24 cases of sickle cell anemia. The second part consists of 10 cases of sicklemia and 2 cases of sickle cell anemia diagnosed before death; the spleens of these cases were perfused by the method described by MacNeal, Otani, and Patterson.[5] Paraffin sections were stained with hematoxylin and eosin, van Gieson's mixture, and by Gomori's [9] silver impregnation method for reticulum. Digestion preparations were also made in some instances according to the method of Kyes.[10]

The 2 patients with sickle cell anemia had enlarged spleens but there was no calcium or extensive iron deposit present and they were therefore considered to be relatively early examples of sickle cell anemia, suitable for study.

Results

Routine Autopsy Sections Stained with Hematoxylin and Eosin. Sections of spleen from 150 cases of sicklemia and 24 cases of sickle cell anemia showed no evidence of congenital or other abnormalities of the arteries or arterial capillaries. "Pooling" in the marginal zone was marked in most cases; in 4 cases of sicklemia no "pooling" was present and the erythrocytes were not sickled, although sickled forms were

found in other organs from these cases. The venous sinuses were generally collapsed and the lining endothelial cells were frequently prominent in their opposing positions. The sickled erythrocytes were compressed and packed, usually in a circular, lamellated fashion with obvious overlapping, giving the impression of considerable rigidity, and they were located in the dilated pulp spaces (Fig. 1).

Arterially Perfused Preparations. Sections prepared from arterially perfused preparations showed the erythrocytes to be washed out of the arteries, arterial capillaries, and the venous sinuses. Erythrocytes were present in the pulp cords, especially in the marginal zones and these obviously represent the "pools" (Fig. 2). In this material it was easy to trace the follicular capillaries, marginal capillaries, and pulp capillaries to their terminations in the pulp cords. There were slight ampullar formations in most instances (Fig. 3), but when compared with normal controls and with the illustrations of others (MacNeal, Otani, and Patterson:[5] Plate 42, Fig. 2; Plate 43, Fig. 4) there was no evidence of abnormal dilatation, congenital defects, or of direct emptying into the venous sinuses. It was impossible, therefore, to explain the "pooling" in the marginal zones by changes in these arterial capillaries.

Preparations Following Venous Perfusion. In sections after venous perfusion, filling of the small venous sinuses with erythrocytes and emptying of the arterial channels were found to have resulted. The pulp cords contained erythrocytes and the "pools" were accounted for by these distended pulp cords. No evidence of reverse flow of erythrocytes from the pulp cords into the arterial capillaries was present. The impression obtained from these slides was that the perfused fluid carried the erythrocytes from the larger veins back into the small venous sinuses; then the fluid escaped from the venous sinuses into the pulp spaces through the stomata of Mollier,[6] and then into the arterial capillary endings in the pulp cords, washing out the erythrocytes originally in these capillaries. There were no demonstrable abnormalities in the arterial capillary terminations or ampullae and, in spite of the reversal of the circulation, no "pools" were produced in the venous sinuses (Fig. 4).

Other Preparations. In 2 cases of sicklemia, the spleens were perfused with distilled water through the arteries until the water became clear, then formol-alcohol was substituted and the spleens were fixed in a distended state. Sections revealed almost total hemolysis of the erythrocytes and the arterial and venous channels were open. No vascular abnormalities were found. The pulp cords were slightly distended but there were no "pools" present (Fig. 5). It was impossible to distinguish the sections of sicklemia cases from sections of normal spleens similarly treated. In spite of the drastic treatment, the pulp cords and other structures were remarkably well preserved.

Sections prepared in the preceding manner and also sections digested in pancreatin [10] were stained for reticulum. No abnormal content or arrangement of the reticulum was seen in the perfused sections and the capillary terminations showed no congenital abnormalities (Fig. 6). In the digested sections, which were not injected, some widening of the pulp cords was present but the results were not satisfactory for interpretation.

Discussion

Rich,[1] in describing the histologic picture of the spleen in sicklemia and sickle cell anemia, said:

"The peculiar splenic lesion characteristic of this condition, and present in latent as well as in active cases, consists in a congenital malformation of the sinuses of the spleen, which permits the free escape of blood into the pulp. The entire pulp may be so involved, but that alone is not specific, as it occurs also in congenital hemolytic jaundice. The anomaly characteristic of sickle cell anemia is the pronounced malformation of the sinuses immediately about the malpighian bodies, leading to the formation of pools of blood partially or completely surrounding the malpighian bodies. This is referred, for reasons discussed, to imperfections in the ampullae of Thoma. There is frequently, besides, an abnormal development of capillaries throughout the malpighian body. The vascular abnormalities in and immediately about the malpighian bodies may or may not be associated with the free escape of blood into the general splenic pulp. The reticulum of the spleen is not primarily abnormal."

Klemperer [4] stated:

". . . . Ono (1930) has not found closed pathways at any period of the embryonal development of the human spleen. In view of this most recent observation, it is questionable whether histogenetic anomalies can be held to account for the circulatory variations. It is important to emphasize this scepticism because of recent attempts to explain the pathology of sickle-cell anemia with the hypothesis that an anomaly of the arterial terminations in the spleen is responsible (Rich, 1928)."

Diggs [2] described the enlarged spleens of sickle cell anemia as showing the splenic cords stuffed with sickled erythrocytes and the sinusoids compressed and empty. He also stated:

"In some cases, but not in all, the capillaries of the malpighian bodies are dilated, so that they appear as multiple small varices or as one or more great pools of blood lying within or at the edge of the lymphoid aggregates."

From the results obtained in this study it is thought that there are no congenital abnormalities of the arterial capillary endings in the red pulp cords (ampullae of Thoma), of the venous sinusoids, or of the pulp cords *per se*.

The histologic picture resembles that of active chronic hyperemia, with the pulp cords, *especially in the marginal zones*, packed with lamellated sickled erythrocytes that do not, because of their shapes, escape readily through the stomata of Mollier.[6] The discharge of concentrated erythrocytes into the pulp cords, from the follicular and marginal capillaries, is accompanied by appreciable arterial capillary pressure which accentuates the packing of sickled erythrocytes and dilatation of the pulp cords in these cases. It is possible that by returning to their normal shape, the previously sickled erythrocytes packed

in the pulp cords would escape through the stomata of Mollier. This is the probable explanation of why more marked changes are not found in the spleens of most cases of sicklemia.

In sickle cell anemia there are always some sickled erythrocytes present *in vivo*.[11] The degree of sickling is variable, and according to some [12] is correlated with the symptoms present. When sickled erythrocytes are always present, as in sickle cell anemia, we could expect to find constantly the "pooling" and intense packing of these sickled forms. This would result, in time, in thrombosis, hemorrhage, and scarring with calcium and iron deposits, and lead eventually to the picture of the small fibrotic spleens found in older, active cases of sickle cell anemia.

Conclusions

1. Histologic examination of various preparations of spleen, obtained at autopsies from people who had sicklemia or sickle cell anemia, failed to show any congenital abnormalities of the arterial capillaries, venous sinusoids, or red pulp cords.

2. The "pooling" in the spleen, described as typical of the sickling phenomenon, occurs in the red pulp cords of the marginal zones.

3. It is thought that the "pooling" is, for reasons discussed, the result of circulating sickled erythrocytes.

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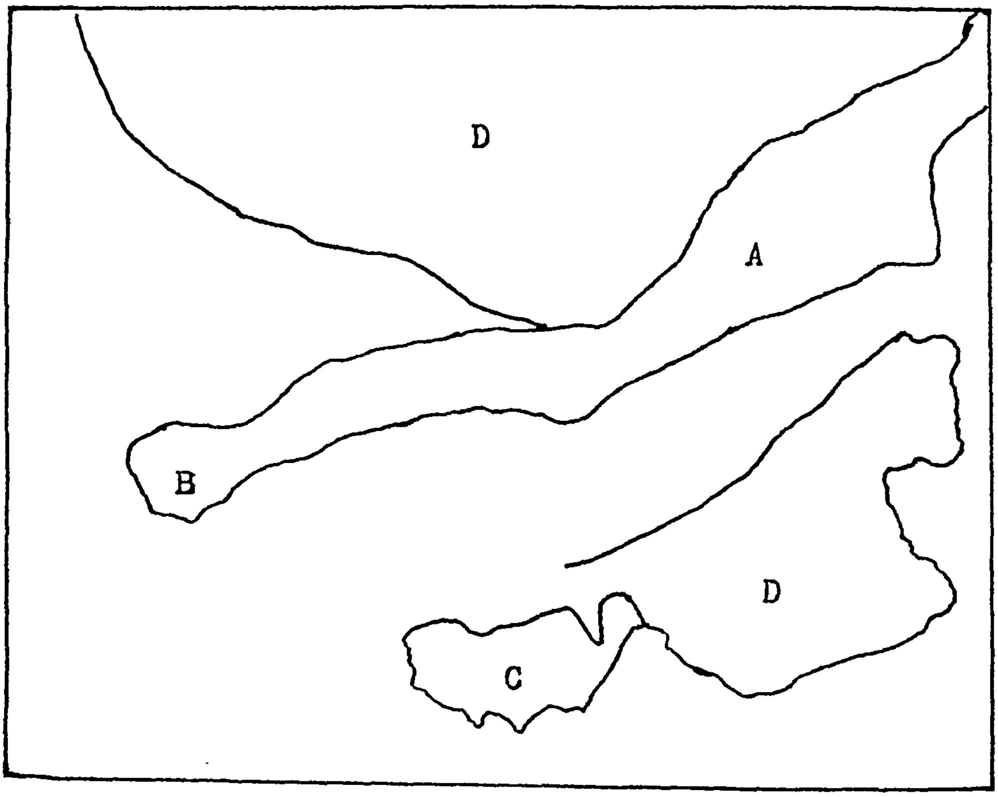


Fig. 1. Sicklemia spleen, not perfused. The follicular capillary entering the marginal zone at A terminates in the pulp cord at B. An empty venous sinusoid is shown at C. The "pools" of packed lamellated sickled erythrocytes are shown at D. Hematoxylin and eosin stain. × 950.

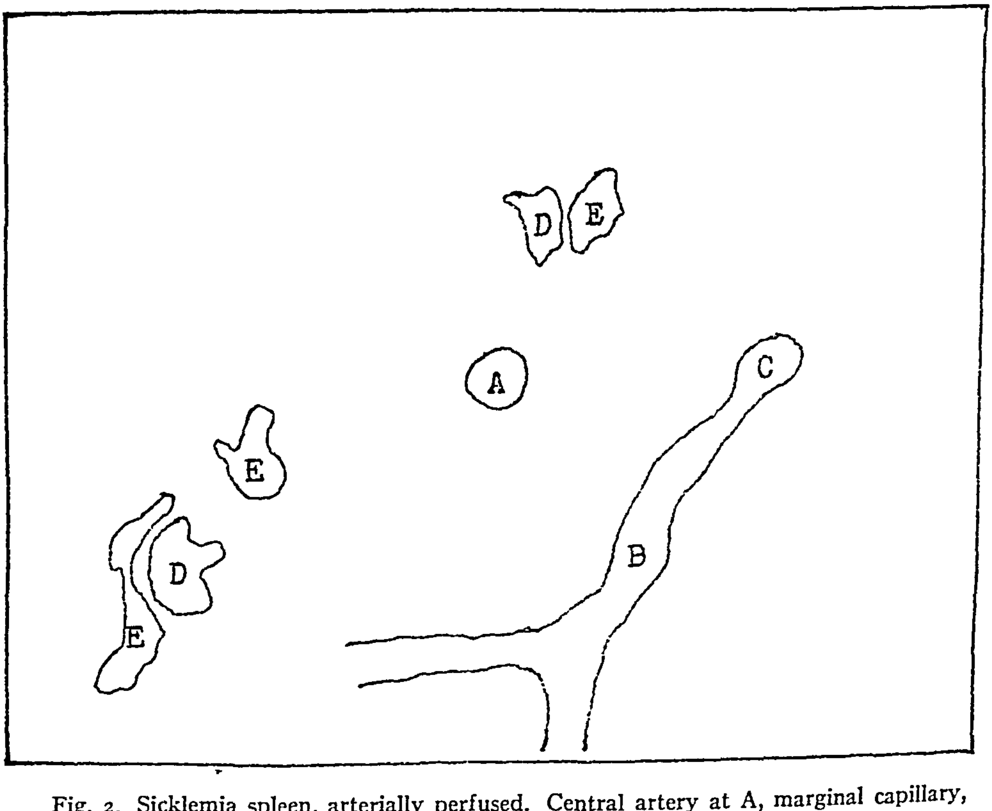
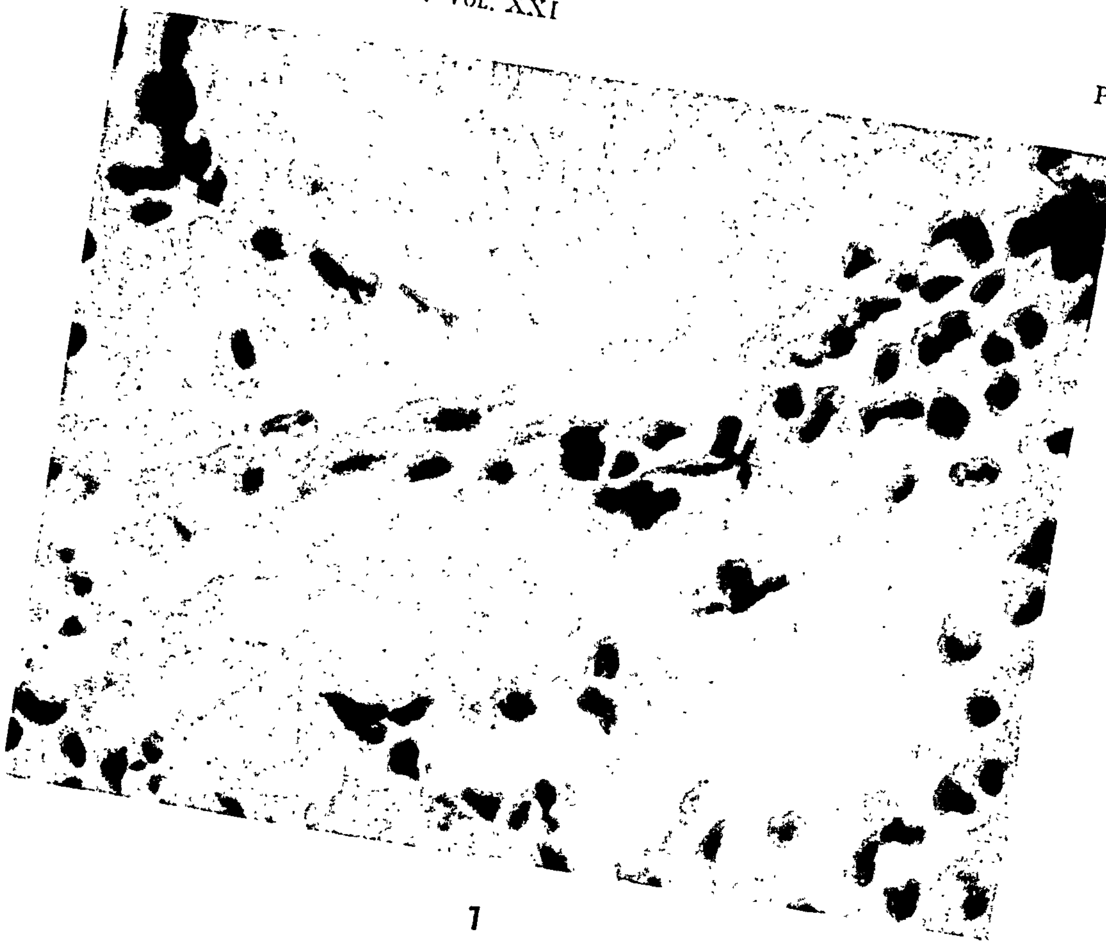


Fig. 2. Sicklemia spleen, arterially perfused. Central artery at A, marginal capillary, B, terminating in pulp cord at C. "Pools" in pulp cords are shown at D and empty venous sinuses at E. Hematoxylin and eosin stain. × 200.



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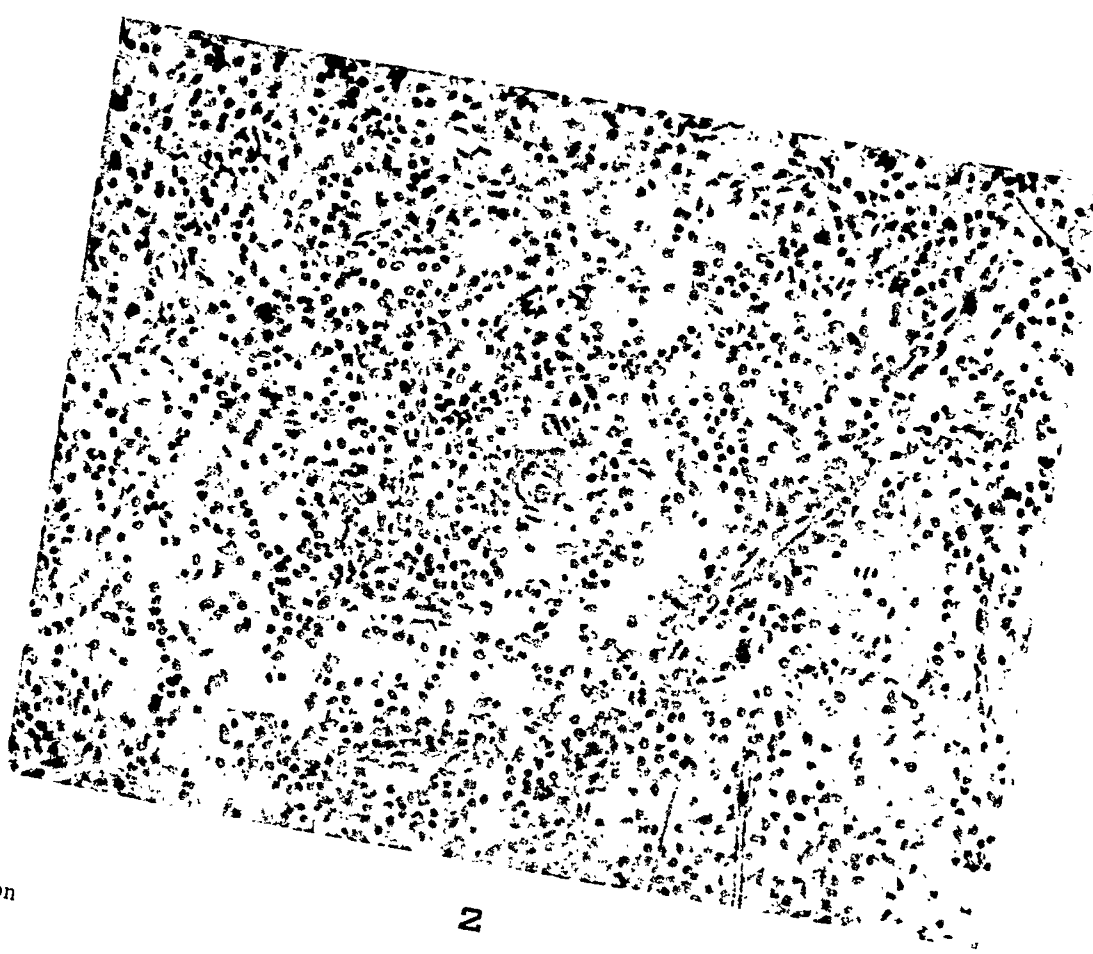

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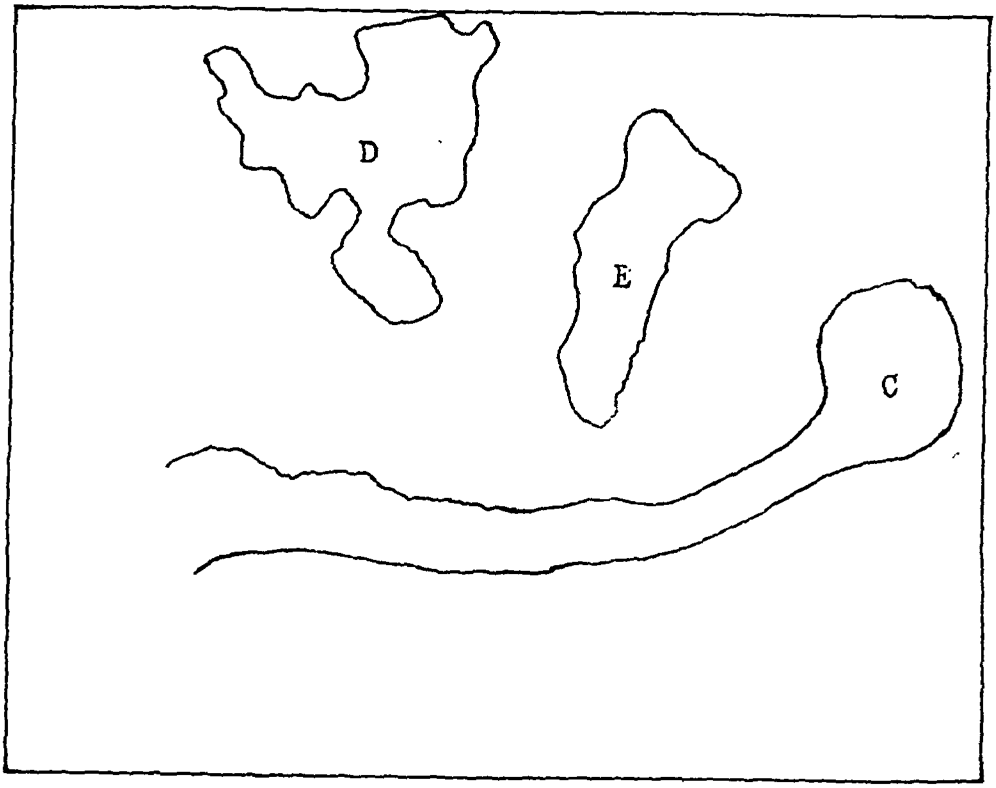


Fig. 3. Higher magnification of Figure 2 showing marginal capillary termination, C, with distention of pulp cords at D and empty venous sinusoids at E. Hematoxylin and eosin stain. × 950.

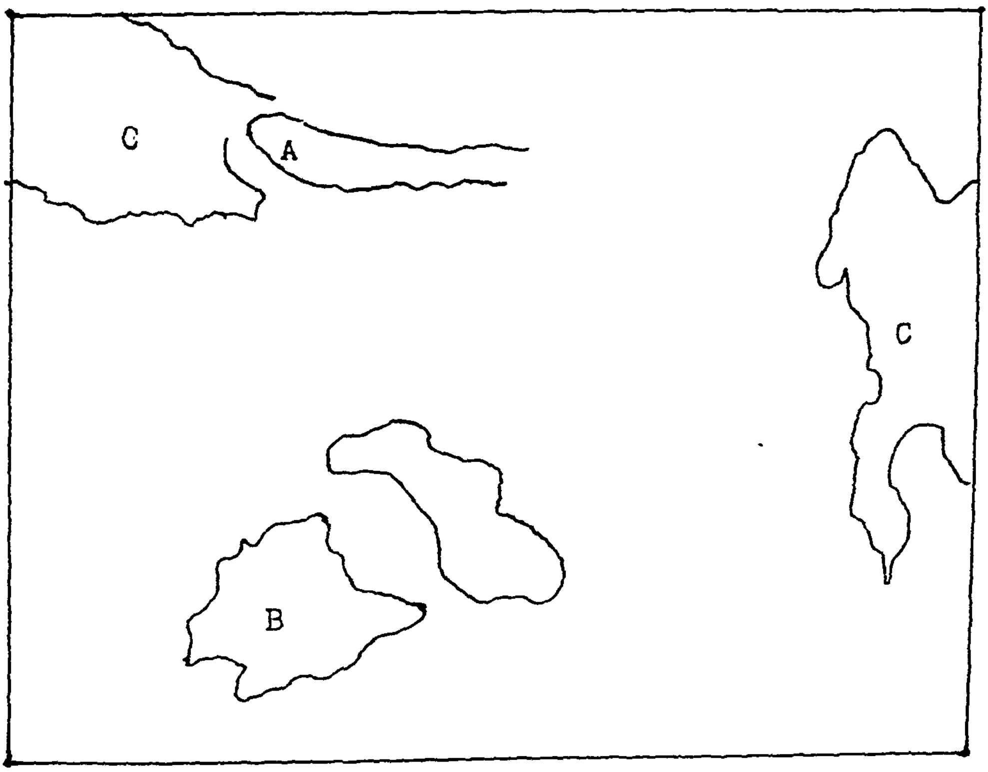
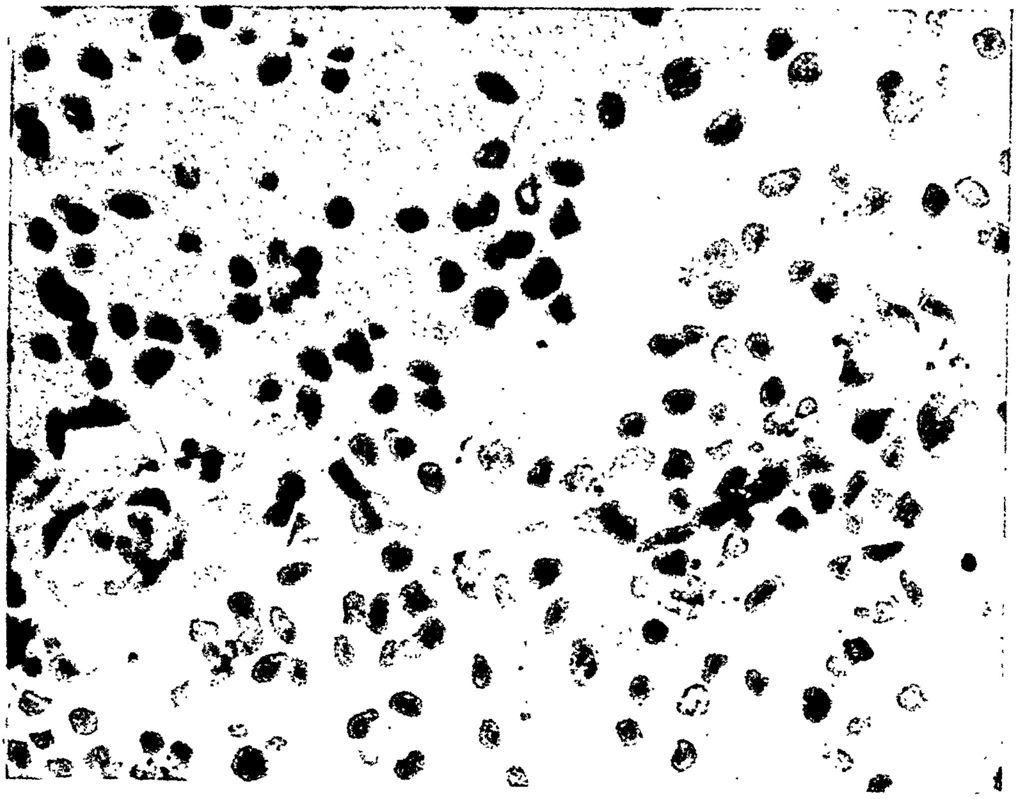


Fig. 4. Sicklemia spleen, perfused through veins. Follicular capillary termination in pulp cord at A, empty venous sinus at B. Distention of the pulp cords is shown at C. Hematoxylin and eosin stain. × 950.



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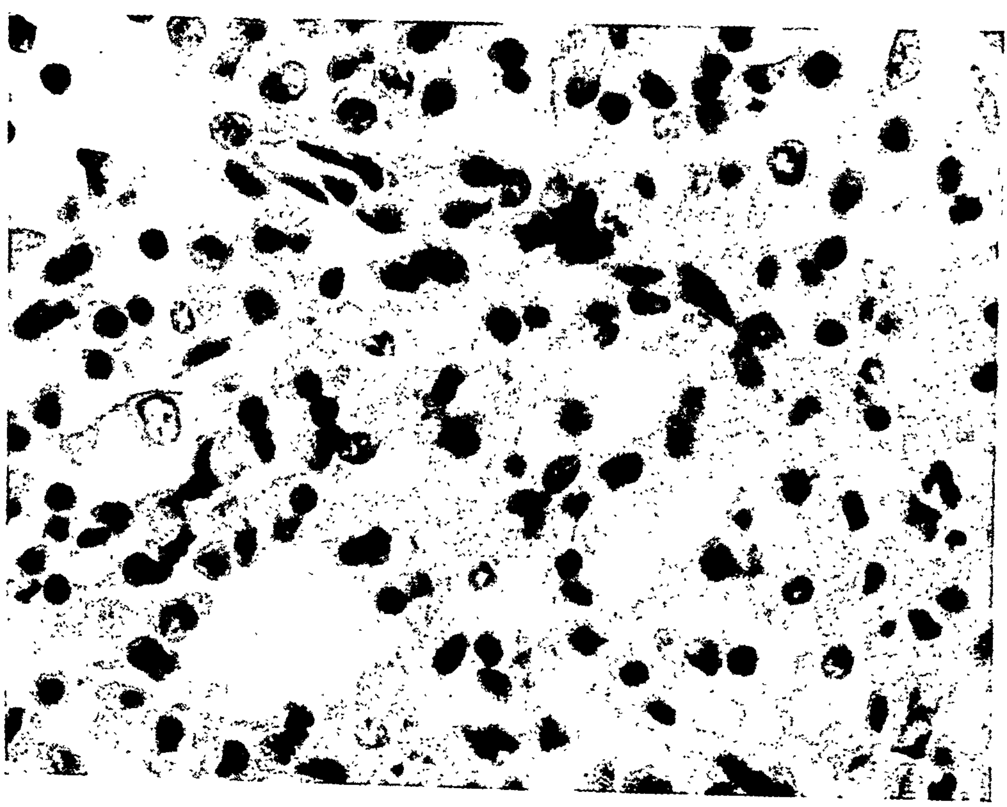
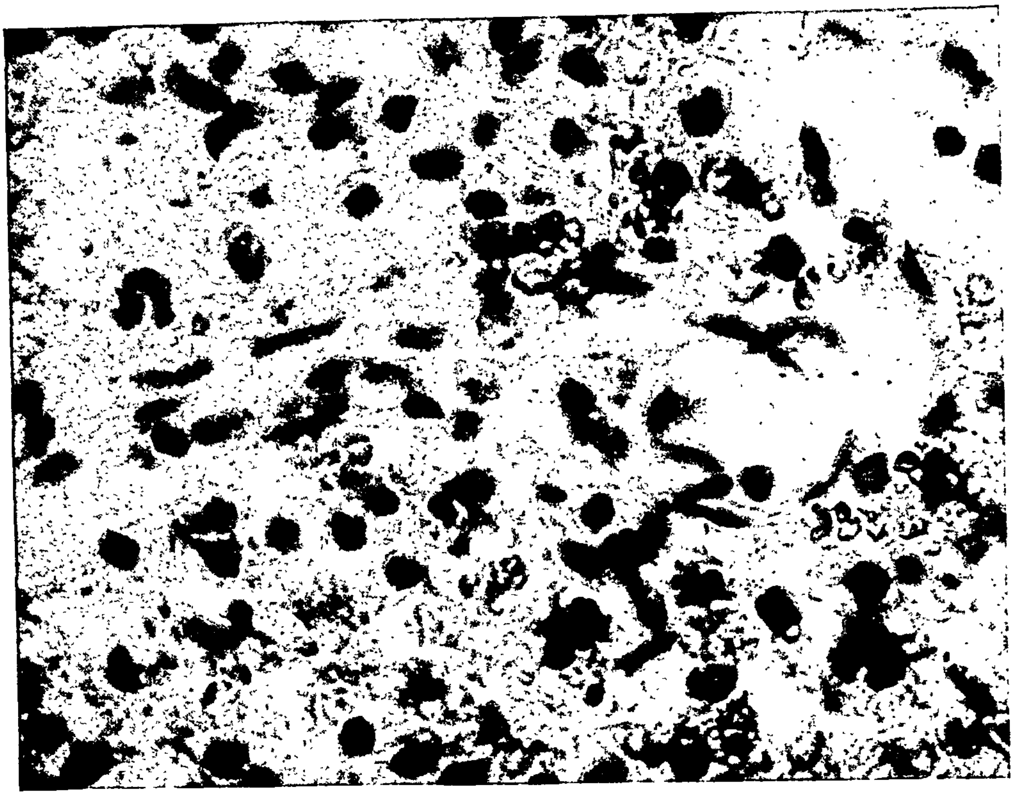


Tomlinson 4 Splenic Circulation in Sicklemia

PLATE 148

FIG. 5. Sickle cell anemia spleen, perfused with distilled water and fixed in distended state. The follicular capillary crosses from left to right, terminating in a slight ampullatous dilatation in the pulp cord. Phagocytized pigment is contained in the reticulo-endothelial cells of the pulp cord. Hematoxylin and eosin stain. × 950.

FIG. 6. Sicklemia spleen, perfused with distilled water and fixed in distended state. The follicular capillary crosses from left to right, ending in an ampulla in the pulp cord. Cells of the malpighian corpuscles are seen in the left half of the field. Gomori's silver stain. × 1000.



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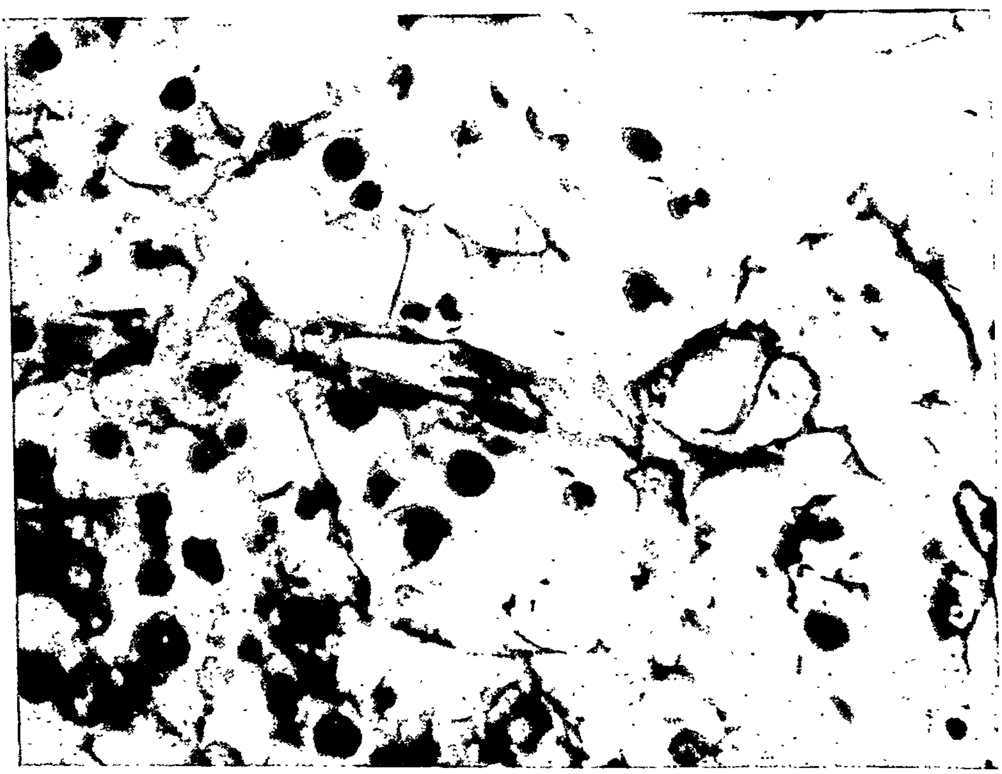

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Tomlinson

Splenic Circulation in Sicklemia

THE MICROSCOPIC DIAGNOSIS OF PULMONARY EMPHYSEMA *

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A still rather widely accepted concept of the nature of the pathologic changes in parenchymal pulmonary emphysema is that of rupture of the walls of contiguous alveoli with coalescence of their lumina into large single spaces. In support of this view, broken portions of alveolar septa terminating in bulbs formed by curling of the severed ends are described as projecting into the air spaces. From a comparison with sections of normal lungs preserved in the state of expansion such an interpretation seems to be erroneous. Although similar doubts concerning alveolar rupture in emphysema have been expressed in the literature, persistence of the idea in a number of standard works on pathology has stimulated this report. An attempt will be made to demonstrate that the significant pathologic changes in simple emphysema are increased size and altered form of the terminal air spaces of the lung. Only in emphysema complicated by formation of subpleural *blebs*,[1] pneumothorax, or pulmonary interstitial emphysema[2] does alveolar rupture occur.

Factors Influencing the Microscopic Appearance of the Lung

The gross and microscopic appearances of advanced stages of emphysema are so characteristic that little need be said about them. In early or doubtful cases naked-eye inspection may be of little help and the diagnosis may rest largely on microscopic examination. Many factors other than emphysema may influence the size and form of the terminal air spaces. Some of these will be discussed briefly.

The lung is a uniquely and exceedingly labile structure. Manipulations incidental to its removal, pressure exerted during palpation, and the effects of slicing the delicate tissue may cause striking changes in the microscopic appearance to a degree impossible in more stable organs such as liver, spleen, and kidney. The usual method of fixation employed at autopsy consists of excising a block of fresh lung tissue and placing it in fixative, commonly 10 per cent formalin or Zenker's solution. This technic, hereinafter referred to as fixation by immersion, does not consistently preserve the tissue at a uniform degree of expansion, for the size and form of the terminal air spaces in sections cut from blocks so treated vary greatly. Manipulation may greatly compress the tissue in some regions and correspondingly distend it in more

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peripheral zones by forcing air, edema fluid, or blood into the alveolar spaces and capillaries. Normal lung collapses when the thorax is opened and the usual picture in immersion-fixed material is that of nonexpansion. Air forced into the subpleural air spaces by proximally exerted pressure or by pathologic alveolar contents such as pneumonic exudates may produce a microscopic picture of expansion and even hyperexpansion in place of the usual collapse. Chronically overdistended lung tissue with diminished elasticity fails to collapse and in immersion-fixed sections also commonly appears expanded, but as emphysema is only one of several factors which can produce this picture, such expansion *per se* cannot be accepted as diagnostic of emphysema. It thus becomes apparent that it is desirable to preserve the lung in some manner which will produce a uniform state of expansion, eliminating extraneous factors so that the distention of emphysema may be accorded greater significance. A useful method is that of introducing fixative into the isolated lungs through the trachea until they have attained a size equal to that of normal inspiration. No matter what method of fixation is adopted, it is necessary to distinguish between the microscopic appearance of expansion in normal and in emphysematous lungs.

The Terminal Air Spaces in Expanded Normal Lungs (Three-Dimensional Consideration)

The anatomy of the terminal air spaces of the lung has been the subject of extensive investigations by Macklin,[3] Miller,[4] Willson,[5] and others. Only a brief account of those aspects considered most pertinent to an understanding of the changes occurring in emphysema is included here.

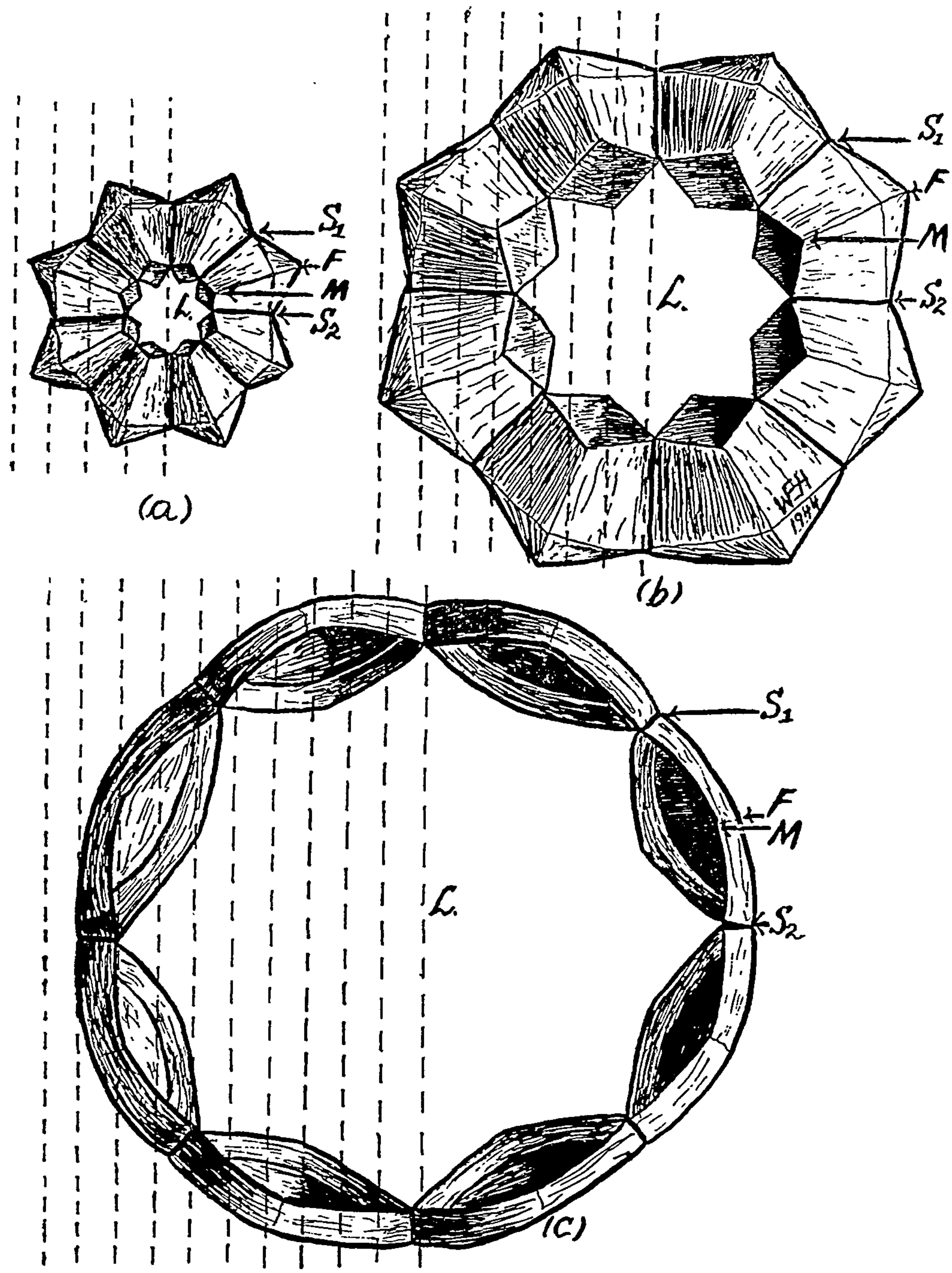
Those subdivisions of the lung subject to significant alterations in emphysema are included in the phrase *terminal air spaces* herein used to refer to alveolar ducts, alveolar sacs, and alveoli. From respiratory bronchioles several *alveolar ducts* arise with walls formed almost entirely by the openings of smaller, blind outpouchings or bays which surround them on all sides (Text-Fig. 1 and Figure 1). These are the *alveoli*. Each alveolar duct ends in one or more slightly expanded terminations, the *alveolar sacs*, similarly surrounded on all aspects by the mouths of alveoli which form their walls. Unless an alveolar duct is cut longitudinally, it cannot be distinguished in microscopic sections from an alveolar sac, for the cross section of a duct has all the appearances of a sac except for a slightly smaller diameter, a difference not sufficiently great for identification. For simplicity the term, alveolar ducts, will be used to refer to both ducts and sacs, for distinction between these two is not relevant to the diagnosis of emphysema. It is,

however, essential that the distinction between alveolar *ducts* and their encircling *alveoli* be clearly envisioned in comparing the terminal air spaces in sections of expanded and collapsed lung. This distinction may be emphasized by comparing an alveolar duct to the central passage of a barn in which rows of open stalls lining both sides represent alveoli. For completeness one must visualize the ceiling and floor as also lined by other open stalls, and the central passage should not be rectangular but approximately circular.

Each alveolus possesses a relatively wide mouth surrounded by a sphincter-like, fine band of smooth muscle,[3] and side walls which slope gently outwards to a base slightly larger than the mouth. The alveolar bases which intervene between neighboring ducts are most frequently in the form of cones, the central point being located most distant from the alveolar mouth.

The Appearance of the Terminal Air Spaces in Sections of Normal Lung Preserved in the State of Expansion (Two-Dimensional Appearance)

Figure 1 is from a section of nonemphysematous, adult human lung filled with Bouin's picric-formol solution introduced under moderate pressure into the bronchial tree through a tracheal cannula until the lung attained a degree of expansion comparable to that of normal inspiration. A portion of an alveolar duct (which terminates in an alveolar sac) is illustrated, and the manner in which both are bounded by alveoli is shown. The latter appear as *open* U-shaped outlines, each separated from its neighbor by a common, inwardly projecting septum which ends in a small bulge or knob representing a cross section of the muscle ring which encircles each alveolar mouth. This appearance of an alveolar duct is the result of being sectioned in a plane passing through the central lumen. If, instead, a tangential cut passes through only one side of the duct, the central lumen will not be included in the resulting section and only the row of alveoli forming that wall through which the knife passed will be seen. Since such a cut passes through the alveoli at an angle to their mouth-base axes, the alveolar mouths are "missed," and the alveoli appear in the section as uninterrupted, *closed*, irregularly circular outlines lying next to one another. These two types of alveolar outlines—*open* and *closed*—are not derived from differently constructed alveoli, but are the result of sectioning a single type in different directions. *Zig-zag* lines [6] are another feature to be noted in sections of expanded normal lung. They are formed wherever a series of sectioned conical bases separating alveoli of neighboring alveolar ducts are seen. Each base appears V-shaped in outline and dovetails with others in a manner somewhat suggesting the cells of a honeycomb. A row of alternating V's produces a *zig-zag* line.



Text-Fig. 1. This is a diagrammatic representation of eight alveoli surrounding the lumen (L.) of an alveolar duct in cross section at various stages of expansion. For the sake of simplicity the alveoli are represented as four-sided whereas actually they are probably more often octahedral. The dotted, parallel, vertical lines represent a series of random sections passing through each three-dimensional figure.

(a) Depicts the alveoli in a partial degree of collapse. The alveolar dimension from mouth to conical base is greater than width or diameter taken at right angles to the mouth-base depth. In the series of sections indicated by the dotted lines only one would pass through the lumen of the alveolar ducts and by cutting the alveolar mouths produce *open* alveolar outlines.

(b) Depicts the alveoli in a state of expansion comparable to normal inspiration.

The alveolar depth and width, as defined above, are nearly equal. The sections indicated by the dotted lines would include the lumen of the alveolar ducts in about half the cases and by passing through the alveolar mouths *open* alveolar outlines would be produced. In the remaining half the sections would contain only *closed* outlines.

(c) Represents the alveoli in emphysema. Alveolar diameter or width greatly exceeds depth as measured from mouth to base. Nearly all the sections indicated by the dotted lines would pass through the lumen of the alveolar ducts, and in only one would *closed* outlines be seen. By comparing (a) and (c), the manner in which the greater proportion of air-containing space changes from alveolar to alveolar ductal in nature may be seen.

In each of the diagrams, S_1 and S_2 indicate comparable positions on the alveolar septa; M, the alveolar mouth; and F, the floor or base. As the alveolar diameter indicated by the distance between S_1 and S_2 increases, reaching a maximum in emphysema (c), the alveolar depth measured between M and F decreases. The *ratio* of depth to alveolar circumference thus decreases as the terminal air spaces are progressively expanded.

The Terminal Air Spaces in Normal Lungs in the State of Collapse (Three-Dimensional Consideration)

In sections of lung obtained at autopsy the terminal air spaces are most commonly observed in varying degrees of collapse. As the air leaves the alveoli their side walls come closer together, the circumference diminishes, and depth as measured from mouth to base increases. Their shape thus changes from that of a cup to that of a test tube. Since the circumference of a duct is the sum of the diameters of the mouths of the alveoli forming its boundary in any one plane, this decrease in the diameter of a collapsed alveolus is accompanied by a corresponding diminution in the duct circumference. This is illustrated diagrammatically in Text-Fig. 1-a. The chief characteristics of a collapsed alveolus are thus increased depth and decreased circumference with a corresponding diminution in the size of the parent alveolar duct. Due to their elasticity, there is also an actual contraction of the alveolar walls and bases.

The Appearance of the Terminal Air Spaces in Sections of Normal Lung Preserved in the State of Collapse (Two-Dimensional Appearance)

Figure 2 is of a section of lung which has been preserved by immersion in fixative with no precaution to prevent collapse. The terminal air spaces are much smaller, but even more significant is the change in the alveolar proportions. In addition to an actual decrease in the capacity of the alveolar spaces, a change has occurred in the ratio of alveolar depth to circumference, the former increasing and the latter decreasing. As a result of the small size of the lumen of the alveolar duct (Text-Fig. 1-a), and the relative increase in alveolar depth, random sections do not pass through the alveolar mouths as often as in expanded lungs. *Open* alveolar outlines are thus relatively rare and *closed* outlines are correspondingly more frequent in sections of collapsed than in those of expanded lungs. The *zig-zag* lines formed by

the rows of contiguous alveolar bases in sections of collapsed lungs are prominent, presenting a serrated appearance.

The Terminal Air Spaces in Emphysematous Lungs (Three-Dimensional Consideration)

Emphysema is essentially a condition of hyperexpansion of the terminal air spaces. Increase in size and alteration of the form and proportions of the alveoli and alveolar ducts are the cardinal features (Text-Fig. 1-c). With this expansion there is an absolute increase in the area of the alveolar septa and bases produced by stretching the elastic membranes forming them. To facilitate a maximum increase in the alveolar circumference there is also a decrease in the alveolar depth as measured from mouth to base. The total area of the side walls of any alveolus is the product of circumference times depth, and as the former increases the latter must decrease in a manner comparable to the narrowing of a rubber band in the direction at right angles to that in which it is stretched. In an alveolar septum this change is made possible by virtue of its unattached free border surrounding the mouth which retracts towards the base, thus diminishing the actual depth as well as the ratio of depth to circumference, for the latter increases as depth decreases (Text-Fig. 1). Compared to the side walls the alveolar base is subject to greater stretching for it is attached at every border to the surrounding septa, but its expansion is facilitated by an almost complete loss of its conical form which becomes flattened. The lumina of the alveolar ducts are increased not only by the expansion of the alveolar mouths, which together represent the duct circumference in any one plane, but also by the decrease in alveolar depth which thus encroaches to a much less degree upon the central duct space. The results of these changes are an absolute increase in the size of the terminal air spaces and the conversion of a large proportion of alveolar space to parent duct space. It is important to note that, as described, this partial conversion of space from small alveolar subdivisions to larger alveolar ducts may occur without actual rupture of the alveolar septa. Of physiologic significance is the fact that in hyperexpansion a large proportion of the contained air is thus relatively distant from septal capillaries, whereas in normal expansion nearly all the air in the respiratory portion of the lung is in fairly close proximity to capillaries.

The Appearance of the Terminal Air Spaces in Sections of Emphysematous Lungs (Two-Dimensional Appearance)

In sections of emphysematous lung preserved routinely by immersion in fixative, the tissue nearly always appears in an expanded condition. Figure 3 is a photomicrograph illustrating emphysematous

lung preserved by the method used for that shown in Figure 1. As a result of the diminished alveolar depth, the alveolar septa project for only a short distance into the enlarged lumina of the alveolar ducts. The dimension, depth, taken approximately at right angles to the diminished mouth-base line, is increased as indicated by the enlarged circumference. The loss of the V-shapes of the alveolar bases incident to their stretching has smoothed out the *zig-zag* lines. Due to their shallow saucer-shape, few alveoli have been sectioned in planes which do not pass through their wide mouths and a high proportion of the resulting outlines are *open* rather than *closed*. The characteristic microscopic features of such sections thus include short alveolar septa, wide alveolar mouths, bases which join to form smooth rather than zig-zag lines, a predominance of open alveolar outlines over closed, and an inclusion by alveolar ducts of a large part of the space formerly bounded by alveoli. The increase in alveolar diameter taken at right angles to depth has produced an increase in the circumference of the alveolar ducts of which they form the walls.

The Microscopic Diagnosis of Emphysema

The characteristics described in the preceding section are easily observed in moderate degrees of emphysema. In sections prepared from advanced cases these features are exaggerated. In the latter instance the process of atrophy is added so that the inwardly projecting alveolar septa may be barely distinguishable or even absent, thus converting the lumina of the alveolar ducts into large, smooth-walled, irregularly circular spaces no longer lined by alveoli. Such an occurrence in the subpleural zone results in a *bulla*.[1]

In incipient or early cases the microscopic diagnosis is not always apparent, and here the need for specific criteria is evident. The greatest difficulty lies in distinguishing sections of only slightly emphysematous lungs from those in which emphysema is absent, but a degree of expansion has been preserved even though fixed by the immersion technic. In emphysema the lung tissue is in a permanent state of hyperexpansion, and collapse is not likely to occur even after death, but persistence of expansion in routine sections of lung, as shown earlier, is not pathognomonic of the condition. An example of non-emphysematous lung in which both a primarily compressed and secondarily distended area is included in the same section is illustrated in Figure 4. In this case the compression was purposefully produced but it demonstrates how such an appearance might be accidentally brought about, for this section is from tissue subsequently preserved in the usual manner by immersion in 10 per cent formalin.

Varying degrees of distention were produced in nonemphysematous lungs by digital compression, as above, and by injection of fixative into the bronchial tree under high pressure, expanding the tissue beyond normal inspiration. Microscopic examination failed to reveal a sufficient decrease in the alveolar mouth-base dimension and in the length of the inwardly projecting alveolar septa to be significant of true emphysema. Marked decrease of the septal height is apparently pathognomonic of emphysematous distention which has been present during life. It was found that even forceful manipulations will not produce this feature. Similarly, uniform obliteration of the *zig-zag* lines has not been produced by technical manipulation after death. These features—shortening of the interalveolar septa and obliteration of the *zig-zag* lines—are therefore offered as specific criteria for the microscopic diagnosis of emphysema.

Incipient Emphysema: a Special Problem

In the first stages of emphysema the condition may conceivably consist of only persistent expansion of the terminal air spaces which no longer undergo the usual degree of collapse during expansion. The change would at this stage be functional rather than anatomical, there being little or no alveolar structural alteration. It may well be argued that examination of the lung at such a stage would not exhibit the features described above, namely, shortening of the alveolar septa and flattening of the bases with obliteration of zig-zag lines. In these cases increased size of alveolar ducts and alveoli may be the only morphologic change. Quantitative estimations demonstrating a significant increase in average alveolar diameter over the normal would then constitute the only microscopic criterion upon which to base a diagnosis. Such estimations are too laborious and time-consuming for practical application. Methods involving preservation of the lung at carefully controlled degrees of expansion, and estimation of specific alveolar dimensions in sections 25 μ thick prepared therefrom, have been described [7] for the normal. In the absence of quantitative studies on specially prepared sections, I am unable to suggest other, more easily, demonstrated features pathognomonic of emphysema in its early stages and sufficiently characteristic for diagnostic purposes. Further studies may provide new light on this problem. In this connection changes in the elastic tissue have been suggested and these will be briefly considered.

Elastic Tissue in Emphysema

It is generally realized that the elastic recoil of normal lung tissue is absent or diminished in emphysema. This is doubtless associated with the chronic state of hyperexpansion of the terminal air spaces which is

the characteristic feature of the condition. Whether this diminished elasticity produces the condition or is its result is outside the scope of this paper. In long-standing cases, elastic tissue cannot be demonstrated or is markedly reduced throughout the alveolar walls in the affected regions. Here, however, the altered size and proportions of the alveoli are so characteristic that no further features are necessary to establish the diagnosis. In earlier cases I have not observed any demonstrable decrease in the elastic fibers. In the diagnosis of early emphysema from microscopic sections, elastic tissue stains are thus of little practical aid.

Alveolar Rupture

If the evidence for alveolar rupture in emphysema, referred to in textbooks of pathology, be considered in the light of the microscopic appearance of expanded normal lung tissue described earlier, it is apparent that the so-called ruptured septa are merely the free ends of open alveolar outlines. The textbook figures illustrating broken portions of alveolar septa terminating in bulbs, thought to be formed by curling of the severed ends, are actually those of normal, inwardly projecting septa terminating in the bulge caused by cross sections of the smooth muscle surrounding the alveolar mouths.[8] Such alveolar septa constitute the side walls of *open* alveolar outlines. Many pathologists are familiar with sections of the normal lung only in a state of collapse. In such sections the majority of the alveoli appear as *closed* outlines, as previously explained, and the muscle bundles surrounding the mouths of any *open* outlines which may be present are not apparent, for the entire wall in the relaxed, collapsed stage is as thick as the terminal muscle bundle. Since *open* outlines are few in sections of collapsed lungs, the large number seen in expanded lungs might easily lend credence to the view that they represented *closed* outlines which had been ruptured and thus converted from the original O-shape to a U-shape. This is the result of considering the finer subdivisions of the lung only in a two-dimensional sense, as seen in sections, instead of correlating the appearances of these thin slices with that of the original three-dimensional alveoli and alveolar ducts. It is hoped that such correlation as has been attempted here will lead to the more general acceptance of shallow, wide alveoli and smoothing of the *zig-zag* lines formed by their bases as the microscopic criteria of emphysema, rather than alveolar rupture with coalescence of their lumina into single large spaces.

It is not denied that severe stretching of the alveoli may sometimes produce rupture, but the most likely site for occurrence is not the alveolar side walls but the alveolar bases, particularly those abutting against either the pleura or vascular sheaths. Pores (Macklin [9]) are

absent in such bases which are thus subjected to increased pressure only from one side. Rupture at these sites results in the formation of pleural *blebs* [1] and possibly pneumothorax, or, in the case of alveolar bases lying against vascular sheaths, produces pulmonary interstitial emphysema.[2] The emphysematous condition is then no longer uncomplicated, and such sequelae do not come within the scope of this paper.

CONCLUSIONS

1. Evidence has been presented to show that in uncomplicated emphysema rupture of the alveolar walls is rarely seen. Appearances suggestive of this in microscopic sections are in reality those of open alveolar outlines produced by sectioning the terminal expanded air spaces in planes passing through the mouths of the alveoli forming the walls of alveolar ducts.

2. Three microscopic features of emphysematous lungs are suggested as diagnostic criteria: (a) marked decrease in the average alveolar depth; (b) a corresponding increase in average alveolar diameter; (c) flattening of the alveolar bases.

3. Quantitative methods for estimating small increases in average alveolar diameter are suggested for the recognition of emphysema in early or doubtful cases.

4. If rupture occurs, the emphysematous condition is no longer uncomplicated. The most common site for rupture is at alveolar bases abutting against either the pleura or vascular sheaths. This may result in pleural *blebs* or pneumothorax in the first instance, and pulmonary interstitial emphysema in the second.

I wish to thank Professor William Boyd of the Department of Pathology, University of Toronto, for his criticism and aid in the preparation of this paper.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 149

FIG. 1. Section of normal, adult human lung preserved in the state of expansion by intrabronchial filling with fixative. Open alveolar outlines and zig-zag lines between neighboring alveolar ducts are well shown. $\times$ 36.

FIG. 2. Normal, adult human lung preserved by immersion in fixative. The air spaces are partially collapsed. The small size of these alveoli necessitated a higher magnification than that used for Figure 1 in order that the alveolar form could be shown. The alveolus opening into the lower left corner of the alveolar duct is of test tube form. $\times$ 155.

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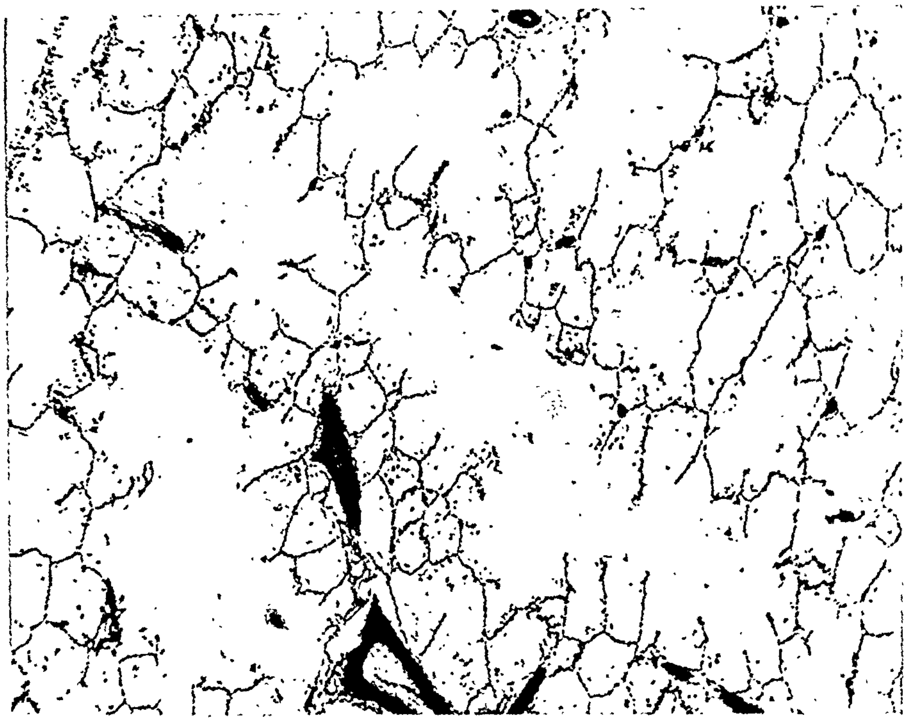

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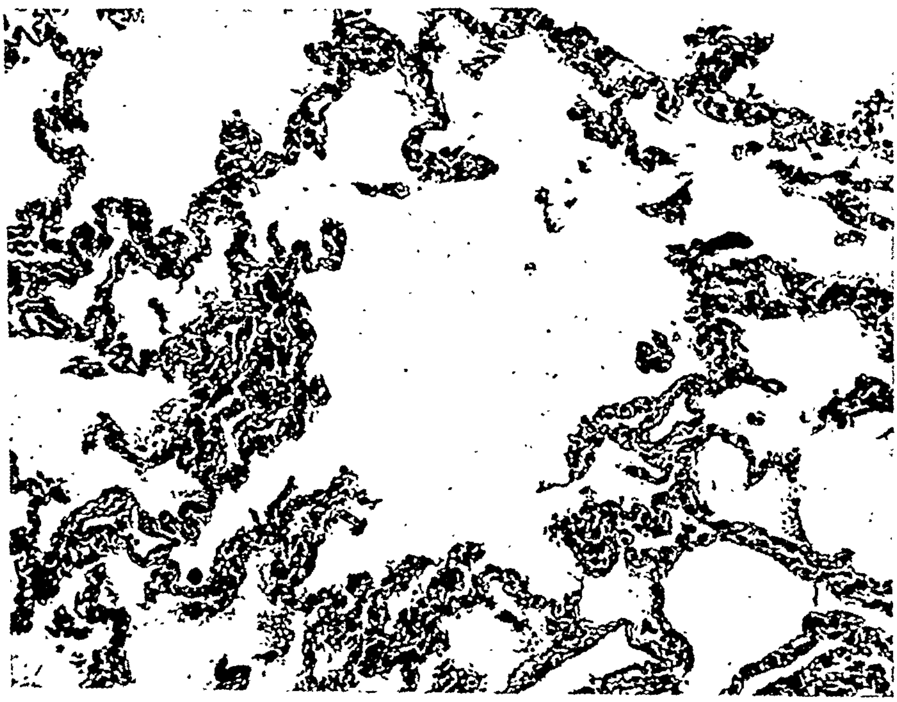

Hartroft		Microscopic Diagnosis of Pulmonary Emphysema

PLATE 150

FIG. 3. Section of adult human, emphysematous lung preserved by the same method employed for the lung of Figure 1. As compared with Figure 1, increased breadth and shallowness of the alveoli and smoothing of the zig-zag lines are shown. × 36.

FIG. 4. Normal, adult human lung illustrating how manipulation may produce compression in one region and distention in adjoining peripheral portions of the tissue. Lung preserved by immersion in fixative. × 5.

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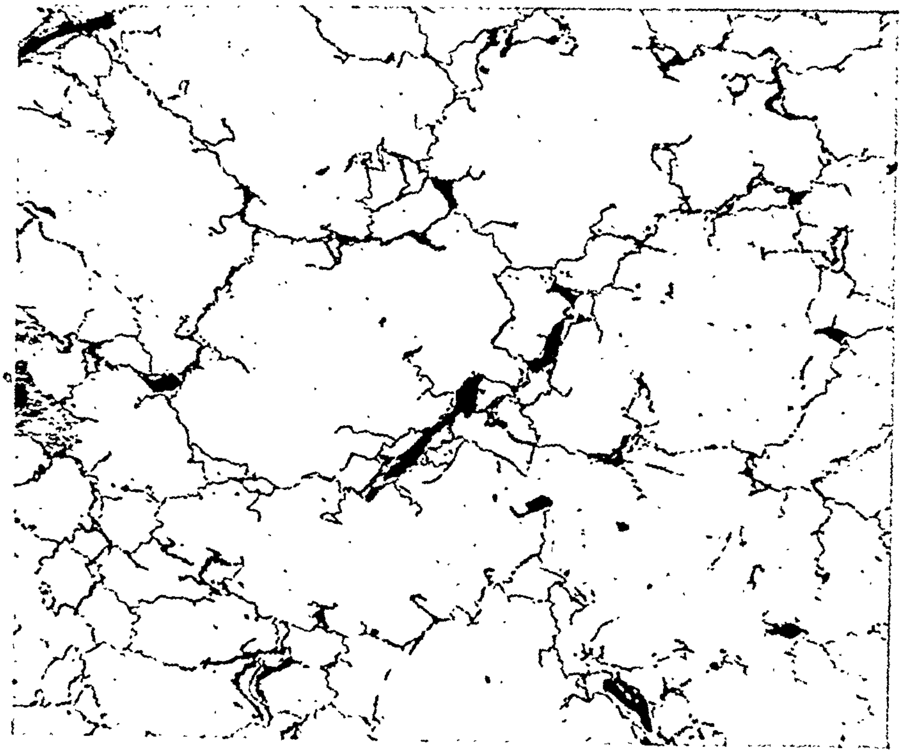

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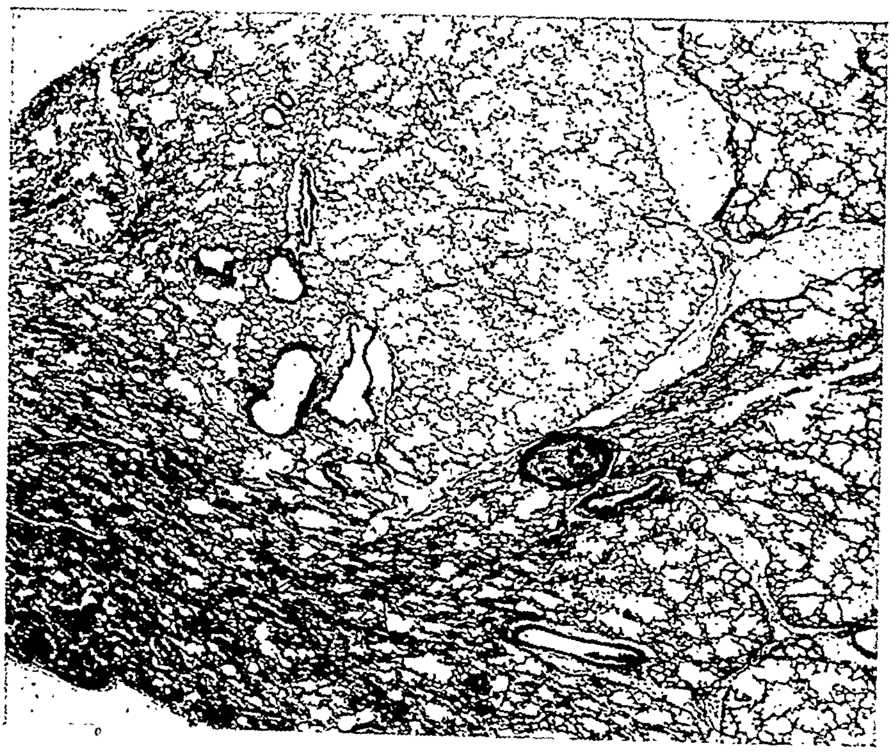

Hartroft

Microscopic Diagnosis of Pulmonary Emphysema

THE VISCERAL LESIONS IN MEASLES

With a Report of Koplik Spots in the Colon *

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Although lesions of the lymphatic tissues of the abdomen in measles have attracted much attention, intestinal Koplik spots have been described but once. Hobson, who was unable to secure material for microscopic examination, observed them in a colostomy stoma. Others have commented on the possibility of a general intestinal response. Newman and Milstead noted bluish discoloration of the cecum during appendectomy in a case in which the lesions of measles were later demonstrated in the appendix. Stryker found Warthin-Finkeldey giant cells in Peyer's patches and Herzberg suggested that there might well be widespread lesions of the lymphoid tissue elsewhere than in the appendix.

The present report is of a fatal case of measles in which Koplik spots were conspicuous in the colon.

Report of Case

The patient, L. K., was a male child, 22 months old, who had been hospitalized in May, 1943, for acute influenzal meningitis, followed by moderate hydrocephalus, and again in November, 1943, for bronchopneumonia, from which he recovered uneventfully. The rest of his past history was not contributory, and he had never had measles as far as could be ascertained. Two days before the present admission, the child welfare doctor called at the foster home to see the patient's twin brother, who had measles with Koplik spots and a typical rash. L. K. had a slight cough and cold at that time, but no Koplik spots or rash. Two days later, April 19, 1944, he developed a measly rash and convulsions, and was brought to Grasslands Hospital. On admission, he was markedly cyanotic. The convulsions were stopped with sodium phenobarbital and vinethene.‡ He was given an oral airway and frequent aspirations were performed. In spite of these measures, he had difficulty in breathing, cyanosis and convulsions reappeared, and he died 5 hours after admission. The rash disappeared an hour or so before death.

Gross Examination

At post-mortem examination, performed 17 hours after death, a splotchy, cyanotic mottling of the skin was noted over the chest and face, but no evidence of a measles rash remained. Each pleural cavity contained a small amount of clear, straw-colored fluid. The lungs weighed 175 gm., and were slightly emphysematous, with moderate congestion at the bases. The cut section revealed a moist, bright sal-

* Received for publication, November 30, 1944.

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‡ A proprietary form of vinyl ether, phenyl alphanaphthylamine.

mon pink surface with slight mottling at the bases. The trachea and bronchi contained a small amount of yellowish white mucoid material.

The thymus was not unusual in size or appearance. The peribronchial and paratracheal lymph nodes were slightly enlarged.

The esophagus, stomach, duodenum, and rectum were not remarkable grossly. The Peyer's patches of the ileum were bright pink, sharply circumscribed, and hypertrophied. Extending from the base of the cecum to 6 inches below the ileocecal valve, the mucosa of the large bowel contained, in gradually decreasing number, many slightly elevated pink areas, 5 mm. or less in diameter. The center of each areola was marked by a slightly depressed, gray punctum of pin-point size. The intervening mucosa was not visibly inflamed, but the thickness of the intestinal wall was slightly increased by edema. The appendix appeared to be normal. The mesenteric lymph nodes were soft, from 0.5 to 1.5 cm. in diameter, and gave the mesentery a gray, nodular appearance. The cut section of these and the thoracic nodes showed a fairly homogeneous ivory white surface.

The heart, liver, spleen, kidneys, adrenals, and other organs were not remarkable except for various degrees of congestion. Only the spleen contained noteworthy microscopic lesions. Chronic adhesive arachnoiditis and hydrocephalus were present but they are not germane to this discussion. No signs of acute disease, other than vascular congestion, were noted in the central nervous system either grossly or microscopically.

Microscopic Examination

Microscopic examination of the lungs revealed a moderately severe interstitial pneumonia, with a mononuclear cell infiltration in the bronchial walls and surrounding alveoli, and an acute emphysema. The most interesting microscopic changes were seen in the epithelium of the bronchi and in the peribronchial lymphatic tissue.

The epithelial change was a peculiar fusion and exfoliation of wide sheets of epithelium into the lumina of the bronchi and bronchioles. The cilia were preserved, but the nuclei of the epithelial cells were gathered into central grape-like masses, showing hyperchromatism, pyknosis, and karyorrhexis (Fig. 1). The cell boundaries were indistinguishable and the cytoplasm was homogeneous and strongly eosinophilic. The denuded bronchial walls showed regeneration of epithelium, and in some areas, where sloughing was not marked, squamous metaplasia (Fig. 2). These changes involved the bronchial tree to the terminal bronchioles, and, rarely, these fused epithelial sheets were found loose in the alveoli. In addition, the lumina of the bronchioles and bronchi contained a small amount of exudate consisting of leukocytes, fibrin, and large vacuolated mononuclear round cells.

The other change was the formation of giant cells of the Warthin-Finkeldey type in the peribronchial lymphoid tissue (Fig. 2). These were not numerous, but each section of lung contained a few. The giant cells were mulberry shaped, with 5 to 20 dark-staining nuclei grouped in a mass in the center or to one side of the cell. The cytoplasm was homogeneous and eosinophilic, with a definite, rounded border. Various stains were used to demonstrate inclusion bodies in the lung and elsewhere, but without success.

The thymus was somewhat hyperplastic. Relatively few Hassall's bodies were present. The most remarkable feature was a multitude of giant cells, so numerous, dense, and hyperchromatic as first to suggest artifacts. They were of the Warthin-Finkeldey type, but rather small and with scanty cytoplasm (Fig. 4). They occurred in fairly distinct lacunae throughout the gland and were most numerous in the cortex of the lobules.

The spleen and lymph nodes of the abdomen and thorax presented a similar appearance. Occasional Warthin-Finkeldey giant cells were seen, tending to occur near the periphery of the secondary nodules in each organ. The secondary nodules also showed central "lymphoid exhaustion" (decrease of lymphoid elements, leaving a rather barren reticulum) and peripheral vascular congestion. The form of the giant cells was similar to that observed in the other organs but the cytoplasm was more abundant than in the thymic giant cells.

The Peyer's patches of the ileum were markedly enlarged, and surrounded by plexuses of distended veins. The center of each secondary nodule showed lymphoid exhaustion, and toward the periphery many contained one or more giant cells. The loose connective tissue adjoining the lymphoid follicles was infiltrated with lymphocytes, plasma cells, tissue eosinophils, and mast cells. The epithelium was thinned and occasionally eroded. Large numbers of giant cells of the specific measles type were found adjacent to the lymphoid nodules, but few within them. They occurred principally in the substantia propria of the villi and none were seen below the mucosa. In general, they were slightly larger than the other giant cells observed, but showed wide variation in size and shape (Fig. 3). The appendix contained analogous lesions, with an exudate in the lumen consisting of small round cells, fibrin, and cellular débris. The walls of both the appendix and ileum were slightly edematous.

The Koplik spots in the colon resembled the lesions of the ileum. Each proved to be an enlarged lymphoid follicle showing central exhaustion, containing a few giant cells, and surrounded at its periphery and in the loose connective tissue of the immediate mucosa by distended vessels. The villi adjacent to the follicles were crowded with giant cells

resembling those in the ileum, and there was similar infiltration with lymphocytes, plasma cells, and mast cells. The edema was slightly more marked than in the ileum (Figs. 5 and 6).

Discussion

Warthin-Finkeldey Giant Cells

The reaction of tissues, other than cutaneous, to the virus of measles has provoked much interest since the simultaneous and independent reports of Warthin and Finkeldey, in 1931, of the presence of giant cells in the tonsils in the prodromal stage of measles. Similar changes had been found in tissues studied by Ciaccio and Alagna in 1910 and 1911 respectively. Alagna, who limited his study to the mucous membrane of the nose and throat, found large multinucleated giant cells in the tonsils, and Ciaccio, observing tissues from the same cases, saw cells resembling megakaryocytes in the newly formed capillaries in the interstitial tissue of the lungs. He also noted swelling of the intestinal lymphoid tissue, but saw no giant cells.

Since Warthin's and Finkeldey's reports, this peculiar, mulberry shaped, syncytial giant cell has been observed in many tissues, and apparently only in association with measles. There has been one report of its presence in the tonsils of a child believed to have chicken pox, but the evidence excluding measles as the diagnosis is rather tenuous.

The relation of Warthin-Finkeldey giant cells to measles has become so widely accepted that Gordon and Knighton used their presence in lymphoid tissue as the criterion of the disease in their studies of the measles virus. In a well controlled experiment, four monkeys were inoculated with blood taken from human patients within 24 hours after the appearance of the measles rash. Lymph nodes were removed from the monkeys on alternate days, and in three monkeys giant cells were found in constant but diminishing numbers from the third to the tenth day after inoculation. None of the animals had clinical signs of measles. The photomicrographs accompanying the report leave little doubt that the giant cells seen were typical. This work also confirms observations in human cases which indicate that giant cells are a manifestation of the prodromal and early acute phases of the disease.

Table I shows the widespread distribution of the Warthin-Finkeldey cells in cases of human measles and will serve as a brief outline of the literature concerning their location and time of appearance. Except as indicated in footnotes to the table, the diagnosis usually was based on the presence of Koplik spots in the mouth, as well as on the systemic signs and an exanthem.

The origin of the specific Warthin-Finkeldey giant cell has been considered by Mulligan, who concluded that they are formed "as a result of the polynuclear abnormal development of the stem cell paralleling the mononuclear normal development of the lymphocyte from the stem cell." Other workers believe them to originate from amitotic division

TABLE I

Reported Distribution of the Giant Cells of Measles and Correlation to the Stage of the Disease

Author	Cases	Location of giant cells	Stage of disease
1. Ciaccio, 1910, and Alagna, 1911	8	Tonsil	Not specified
2. Warthin, 1931	1	Tonsil	4 days before rash
3. Finkeldey, 1931	1	Tonsil	3 days before rash
1932	1	Appendix	2 days before rash
4. Herzberg, 1932	1	Appendix	4 days before rash
5. Davidsohn and	1	Appendix	3 days before rash
Mora, 1932	1	Appendix	2 days before rash
	1	Appendix (rare)	2 days after rash
6. Fischer, 1933	1	Appendix	1½ days before rash
7. Schultze, 1933	1	Appendix	2 days before rash
8. Hathaway, 1935*	1	Spleen, abdominal lymph nodes	Exposed
9. Bullowa, *et al.*, 1937	3	Appendix	Pre-eruptive
10. Gräff, 1937	1	Tonsils, lymph nodes, fossa of Rosenmüller	15 days after exposure
11. Wegelin, 1937	1	Appendix	4 days before rash
	1	Appendix	3 days before rash
	1	Appendix	Day rash appeared
12. Semsroth, 1939†	1	Tonsil, spleen, thymus, lymph nodes, tongue, and bronchi	Exposed
13. Stryker, 1940‡	1	Lungs, tracheal and bronchial mucous glands, lymph nodes, spleen, and Peyer's patches	Exposed
14. Newman and Milstead, 1940	1	Appendix	3 days before rash
15. Mulligan, 1944	1	Appendix	3 days before rash

* This patient was exposed by two older siblings and died without the appearance of a rash or Koplik spots.

† This patient was exposed by two other siblings and died 12 days after the rash appeared in one child, on the day it appeared on the other child. No rash or Koplik spots were present in the case in question.

‡ This patient was exposed in a nursery, presumably.

of plasma cells or lymphocytes, from mesenchymal or reticulum cells, or from fusion of lymphocytes. The last seems the most likely from consideration of our slides, although the large number of plasma cells found in the tissue surrounding the giant cells leads one to suspect that they might be implicated in giant cell formation.

Fused Epithelial Giant Cells

Although there is an almost universal tendency to discount the giant cell of the fused respiratory epithelium type, emphasized by Masugi and Minami, I believe it is as important in the study of this disease as

the syncytial Warthin-Finkeldey giant cell. In some preparations of lung tissues from cases of measles, in which the architecture was destroyed by inflammatory changes, it is impossible to distinguish between the two. Also, occasionally, the epithelium in the intestinal tract shows similar fusion. As far as may be determined in a study of fixed tissues, the transitional stages involved in the formation of both epithelial and lymphoid giant cells, followed by karyorrhexis and pyknosis of their nuclei, are identical. It is probably this kind of cellular response, rather than specific tissue susceptibility, which is characteristic of measles.

MacCallum noted the presence of fused epithelial masses in the lung in his study of pneumonia in army camps during the last war, where measles was the most common predisposing disease, and Stryker also mentioned them. Denton, in 1925, was struck by their appearance and wrote:

"In many bronchioles the wall cannot be made out, for it often shades off into swollen, contiguous alveolar walls. In places, alveoli close to bronchial radicles contain large mononuclear cells from the respiratory epithelium and fused sheets of atypical respiratory epithelium. Numerous giant cells with five to twenty nuclei and well preserved or degenerating cell bodies are present. . . . Dark-staining compact masses of polylobate nuclei, between which no cell boundaries can be seen, lie in some alveolar spaces."

These epithelial giant cells formed a very conspicuous feature in the present case, but were confined principally to the bronchi and bronchioles. Hecht was able to produce extensive and similar lesions by the intratracheal administration of metallic salts and ammonia, which vitiates any conclusions regarding the specificity of these cells for measles, but does not prevent one from suspecting that they may arise from a similar alteration in the cell membrane, whatever the responsible agent may be.

The relationship between the epithelial giant cell and the giant cell of "giant cell pneumonia" in infants and children merits observation. The latter is considered a rare disease most often occurring after measles, and characterized histologically by the presence of giant cells of the Langhans type in the alveoli. Moore and Gross, who considered them to be the result of fusion and hyalinization of exudates, distinguished between them and the fused bronchial epithelial giant cell. Chown believed the giant cell of pneumonia to be the result of metaplasia of alveolar epithelium under the influence of vitamin A deficiency. Whatever the derivation of the cells, they bear more than a superficial resemblance to the bronchial epithelial giant cells which I observed, and may represent a more severe response to the same agent or agents. Although they were found both with and without squamous metaplasia in my case, the possible rôle of vitamin A deficiency acting with a virus may be considered in a study of their pathogenesis. Dietary informa-

tion in my patient is lacking, but the child previously had been admitted to the hospital with evidence of malnutrition.

Inclusion Bodies

In undertaking the report of this case, opportunity to compare the histologic sections with those from his previously reported series was afforded by Dr. James Denton. Similar lesions were noted, varying only

TABLE II

Inclusion Bodies in Measles

Author	System or cell	Type of inclusion	Staining reaction
1. Ewing, 1909	Epithelium of skin and mucous membranes, sweat glands, endothelium	Cytoplasmic, granular, and ring forms in perinuclear vacuoles	Basophilic
2. Mallory and Medlar, 1919	Endothelium of Koplik spots and cutaneous rash	Cytoplasmic, spherical (believed to be cocci)	Basophilic
3. Farber and Wolbach, 1932*	Salivary glands	a. Nuclear, ovoid b. Cytoplasmic	Acidophilic Basophilic
4. Masugi and Minami, 1938	Fused bronchial epithelium, acini of sublingual and salivary glands, buccal mucous glands	Cytoplasmic, round	Eosinophilic
5. Broadhurst, *et al.*, 1937	a. Smears of Koplik spots and nasal mucosa: in basal and columnar cells and lymphocytes	Nuclear (originally)	Nigrosin-staining
1938	b. Blood and tissue cultures: in leukocytes, lymphocytes and fibroblasts	Nuclear (?), granular	Basophilic
6. Semsroth, 1939	a. Giant cells in epithelium of tongue	Cytoplasmic, globoid	Basophilic
	b. Giant cells in tonsil	Cytoplasmic, globoid	Eosinophilic
	c. Fused bronchial epithelial giant cells	Cytoplasmic, globoid	Eosinophilic
7. Goodpasture, *et al.*, 1939†	Epithelium of bronchi, trachea, bronchial mucous glands, and alveolar epithelium	Nuclear, compact but granular	Slightly acidophilic

* Farber and Wolbach observed inclusions in the salivary glands of many infants dying of various causes, one of whom had had measles 4 months previously.

† Goodpasture and others studied five cases of virus pneumonia in children, three of whom had measles. The inclusions were similar in all five cases.

in degree. The principal interest in studying his slides was directed toward inclusion bodies, since none could be found in my material. Before describing my findings, I should like to present a table, which is by no means exhaustive, giving the observations of various workers in this connection (Table II).

Rivers considered the characteristic inclusion body of measles to be cytoplasmic, but attached to it little diagnostic importance.

In three of Denton's cases, I have been able to find eosinophilic,

cytoplasmic inclusion bodies. They were present in the bronchial epithelium (Fig. 7), in fused bronchial epithelial giant cells, in bronchial mucous glands, and in submucous glands involved in the Koplik spots. Inclusions were not found in the above locations in all cases, but each had them in cells of one or more types. The bodies were usually round, homogeneous, of variable size, multiple, and occurred anywhere in the cytoplasm, but generally toward the free edge of the cell. As can be seen in Table II, Semsroth, and Masugi and Minami have reported inclusions of the same type. It is necessary to agree with Rivers, however, that their presence is far from diagnostic of measles, for I have observed similar forms in various epithelia in canine distemper (Green and Evans), and in the nasal mucosa of monkeys with lymphocytic choriomeningitis.

Similar cytoplasmic inclusions have been found in the bronchial epithelium, and zona reticularis and medulla of the adrenal glands of infants dying of "primary virus pneumonitis," as reported by Adams. They differ in being surrounded by a halo, and by appearing singly in the cell. The commonest precursors of this type of pneumonia, according to Adams, are "the common cold, grip, influenza, and measles." The blood of the affected infants in Adams' series was tested only for neutralizing antibodies of influenza virus, and none were found. It would be interesting to know how many of the infants surviving the epidemic subsequently developed measles.

SUMMARY

1. A case of fatal measles is presented in which Koplik spots were found in the colon, associated with large numbers of Warthin-Finkeldey giant cells in the intestine and other organs.

2. The specificity of these giant cells for measles is accepted and the evidence for their widespread occurrence in the tissues of patients in prodromal and initial stages of measles is reviewed.

3. The fused epithelial giant cells found in this case and reported by others are believed to be as important in the study of measles as the syncytial Warthin-Finkeldey giant cells, although probably they are not specific for measles. The giant cells of "giant cell pneumonia" of infants may represent a similar response to the same agent or agents.

4. Search for inclusion bodies gave conflicting results in the material available for study. Similar conflicting observations have been reported by various observers.

I wish to thank Dr. Gilbert Dalldorf and Dr. George Y. McClure for advice and assistance in the preparation of this paper, and Dr. James Denton for the use of his histologic sections of measles material.

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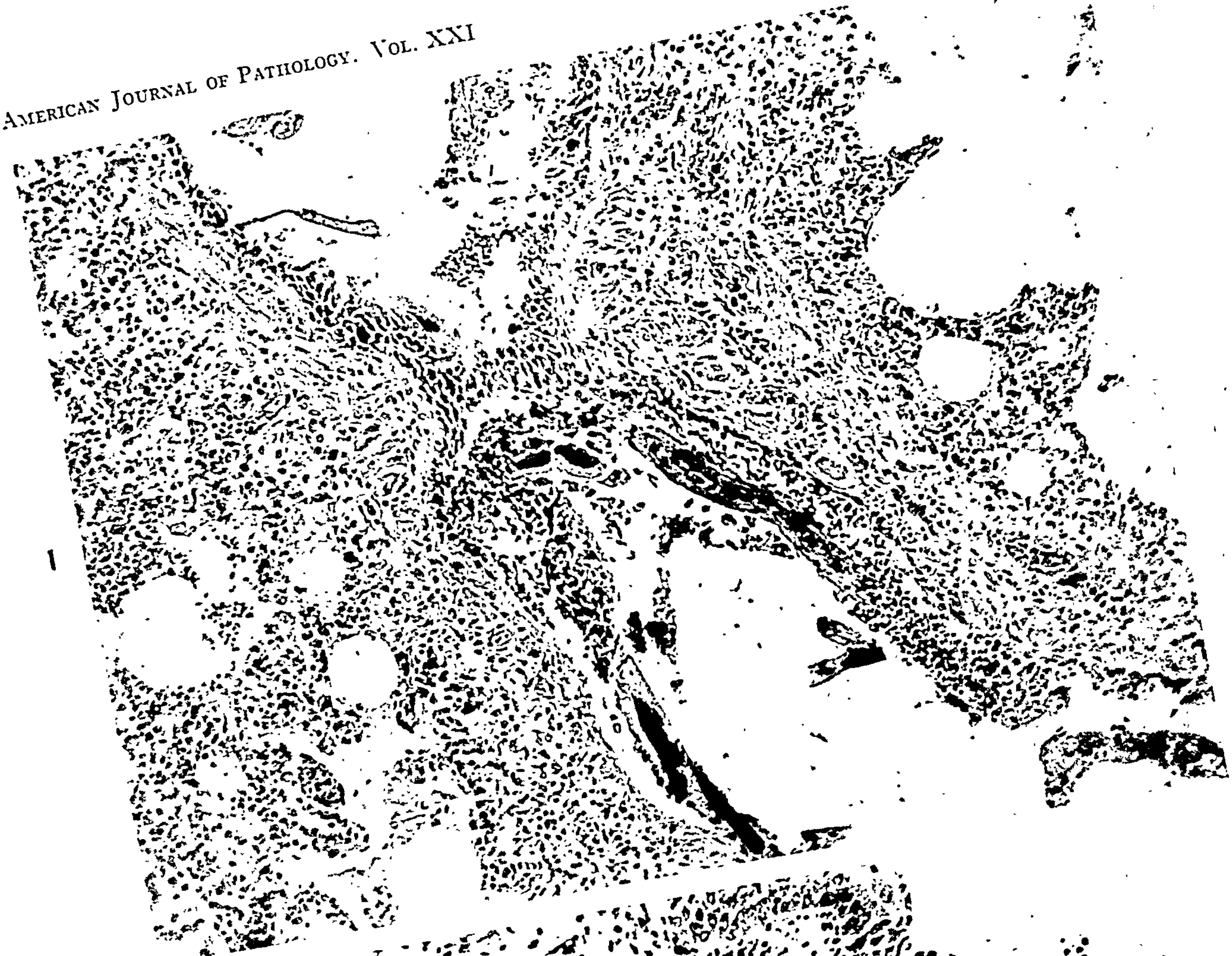
DESCRIPTION OF PLATES

Plate 151

Fig. 1. Bronchus containing fused epithelial giant cells. × 200.

Fig. 2. Bronchus showing squamous metaplasia of the epithelium. A few rather small Warthin-Finkeldey giant cells may be seen in the lymphoid collection to the left. × 307. The inset shows a giant cell from an adjoining field, at a slightly higher magnification. Several basophilic granules were present in the cytoplasm, and one small eosinophilic inclusion was seen in the upper right-hand corner of the cell. (Denton's material.)

1



2

Visceral Lesions in Measles

Corbett

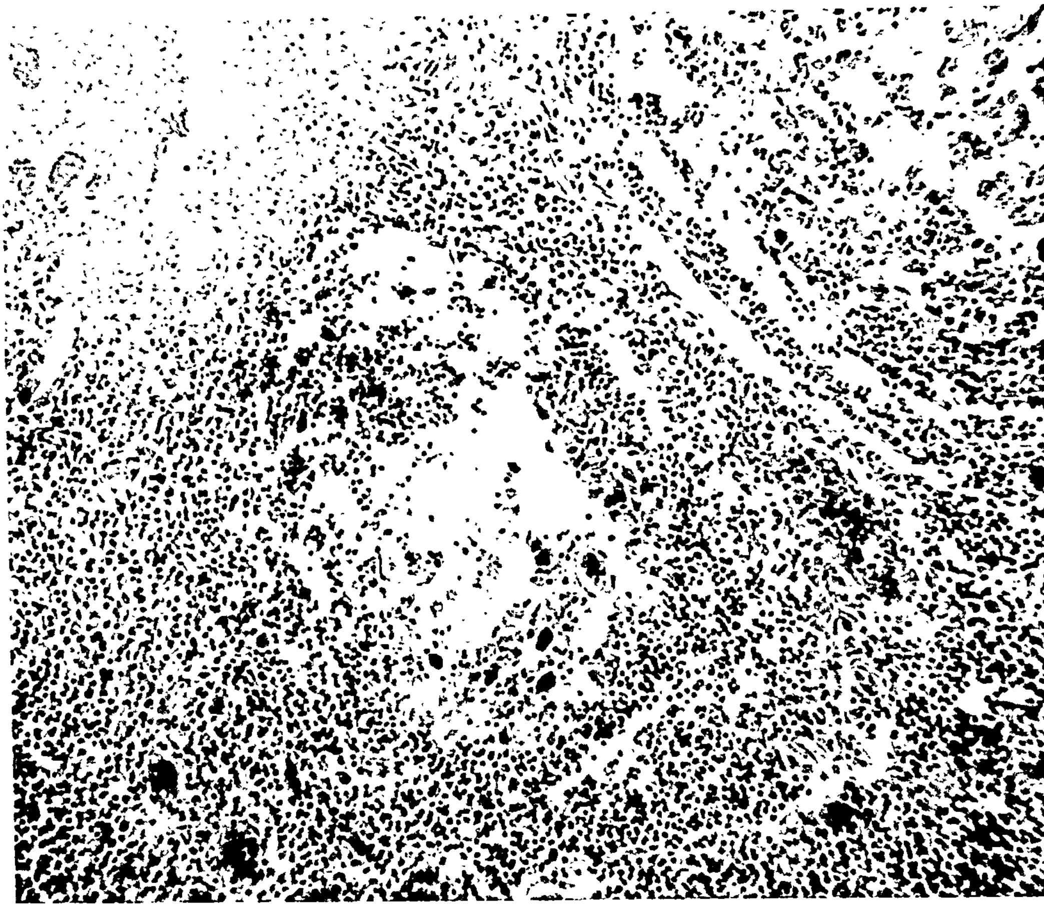
PLATE 152

FIG. 3. Peyer's patch in the ileum, showing necrosis of the epithelium (upper left), central lymphoid exhaustion, and four giant cells. × 307.

FIG. 4. Giant cells in the thymus. × 200.

FIG. 5. Mucosa of the colon adjacent to a Koplik spot, containing giant cells in the substantia propria. × 307.

3



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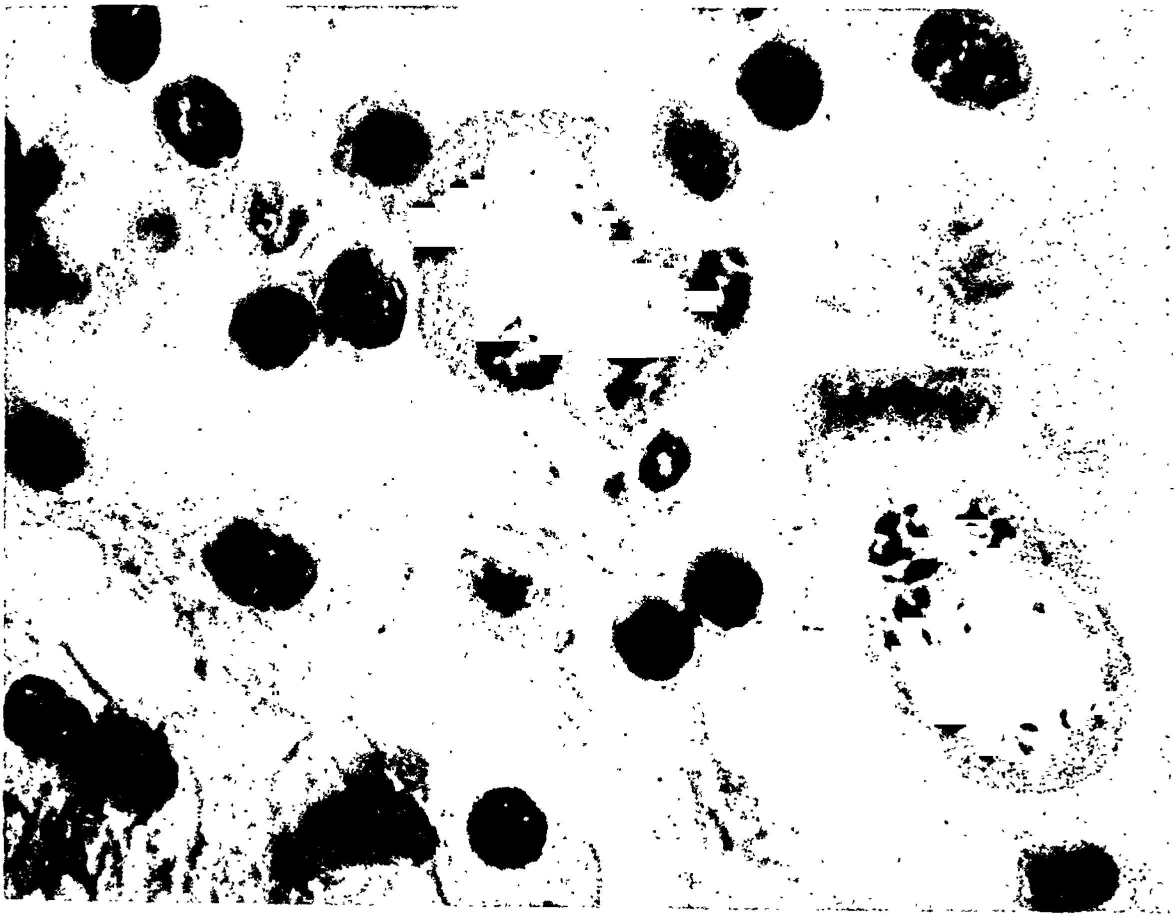
Visceral Lesions in Measles

PLATE 153

FIG. 6. Detail of giant cells in the intestine. × 1820.

FIG. 7. Partially detached epithelial cell from a bronchus, containing four inclusion bodies. × 1820. (Denton's material.)

6



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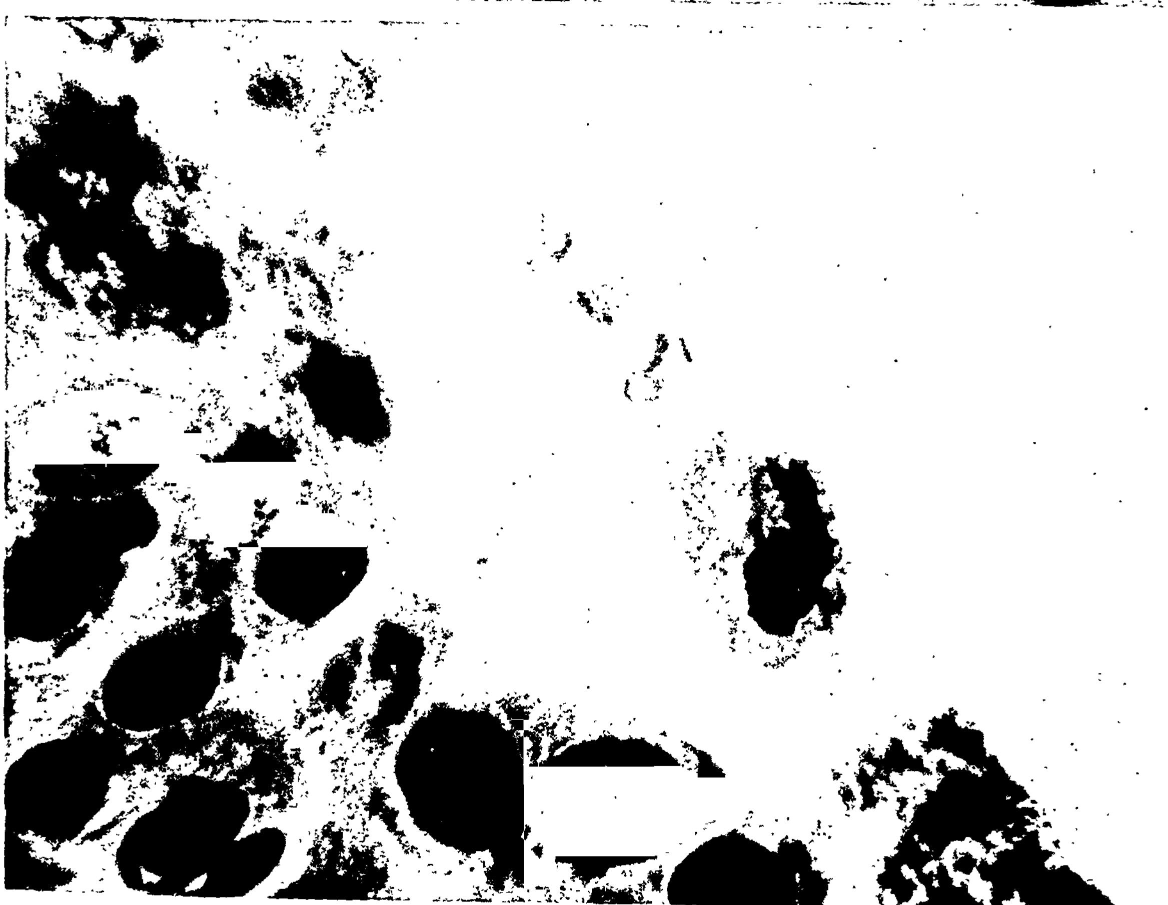

Corbett

Visceral Lesions in Measles

EXTRAGENITAL CHORIOCARCINOMA IN THE MALE *

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Choriocarcinoma of the male is usually primary in the testis. Only rarely does it originate in other situations. Two possibilities exist as to extragenital origin, namely, from elements of teratomas or from embryonal rests of the urogenital fold. Dickson [1] and others have contended that they come from totipotent cells capable of forming trophoblastic tissue which can produce various structures including fetal chorionic tissue. Blastomeres may become dissociated wherever germinal epithelium is found. Arey [2] explained that due to limitation of lateral expansion by the dorsal body wall, the enlargement of the growing mesonephric tubules necessitates longitudinal growth on each side of the dorsal mesentery. Thus the urogenital fold ultimately extends from the sixth cervical to the second sacral segment. The ridge becomes divided into a lateral mesonephric fold and a median genital fold, the anlage of the genital gland. Hence there is a distinct possibility that the primary focus of a choriocarcinoma may be located at the hilum of the lung as a remnant of the urogenital fold persisting in the region of the thoracic segments.

Symeonidis,[3] Prym,[4] and others have emphasized the importance of careful examination of the testes in cases of choriocarcinoma supposedly of extragenital origin. Symeonidis was skeptical of extragenital origin in any case, referred to possible accessory abdominal testes as a source, and maintained that even if derived from a teratoma the tumor originates in trophoblastic tissue.

In Prym's [4] case there were lesions distributed as are metastases of testicular tumors and with a microscopic picture like choriocarcinoma. Because he found a scar, peculiar in its structure and vascularization, in the right testis, he assumed that a primary choriocarcinoma in that testis had undergone spontaneous retrogression. It is said that uterine choriocarcinoma may regress, but in such instances the original diagnosis may have been incorrect. Even if this is true, Prym had no proof that the testicular scar was due to healing of a neoplasm. Nevertheless, these observations indicate the importance of most careful examination of the testes for either tumor or scar in all cases thought to have originated in a situation other than testis. This includes complete study of the genital tract, with serial block sections of the testes.

Adherence to these rigid criteria excludes many cases reported as

* Received for publication, September 25, 1944.

extragenital choriocarcinomas. Among these is that of Bonn and Evans.[5] In this case the primary site was uncertain because a small scar was demonstrated in one testis. Although this scar may possibly have resulted from regression of a tumor, it seems more likely to have been due to an injury sustained 20 years before when the patient was kicked by a horse. Other probable cases * of primary extragenital choriocarcinoma, excluded because of uncertainty about the thoroughness of the examination of the testes, are those reported by Ritchie,[6] Lambert and Knox,[7] Hammarskjöld,[8] Arendt,[9] and Becker.[10] In all of these there were mediastinal teratomas and choriocarcinomatous metastases.

The number of cases, accepted as authentic, varies in different reports. If, however, strict criteria are adhered to and negative serial block sections of the testes considered essential, only 7 of the cases recorded in the literature furnished conclusive proof of an extragenital origin. To these is added the case reported in this paper, making a total of 8 unquestionably proved cases of primary extragenital choriocarcinoma in the male.*

1. Krassnianskaya.[11] Age 72 years. Primary either at hilum of left lung or in retroperitoneal tissue. A pure choriocarcinoma with multiple metastases. No gynecomastia. Testes showed no microscopic change. Aschheim-Zondek test not done.

2. Kantrowitz.[12] Age 22 years. Primary in the superior mediastinum. A complex teratoma in the superior mediastinum with choriocarcinomatous metastases in the lungs. No gynecomastia. Testes showed interstitial cell hyperplasia. Aschheim-Zondek test positive.

3. Fenster.[13] Age 27 years. Primary in retroperitoneal tissue. Pure choriocarcinoma with metastases to lungs and liver. Bilateral gynecomastia. Testes were normal. Aschheim-Zondek test not done.

4. Gerber.[14] Age 23 years. Primary in retroperitoneal tissue, overlying right ileopsoas muscle. Pure choriocarcinoma with metastases to lungs, liver, spleen, and kidneys. No gynecomastia. Testes showed no microscopic change. Aschheim-Zondek test not done.

5. Weinberg.[15] Age 70 years. Primary in urinary bladder. A pure choriocarcinoma with metastases to most of the viscera. No gynecomastia noted. Marked interstitial cell hyperplasia and tubular atrophy in the testes. Aschheim-Zondek test positive.

6. Erdmann, Brown, and Shaw.[16] Age 45 years. Primary in retroperitoneal tissue, posterior to left kidney. Pure choriocarcinoma with metastases to lungs. Enlargement of breasts, considered to be fibroadenomas, probably gynecomastia. Aschheim-Zondek test negative.

7. Hyman and Leiter.[17] Age 57 years. Pure choriocarcinoma, primary in urinary bladder. Bilateral orchiectomy performed. No autopsy. Testes showed no abnormality. Bilateral gynecomastia. Aschheim-Zondek test positive.

8. Laipply and Shipley. Age 21 years. Primary site was a complex teratoma of the superior mediastinum. Choriocarcinomatous metastases in many organs. Testes

* Since this paper was submitted for publication, Plenge[20] has reported a probable case, primary in the retroperitoneal region, and Stowell *et al.*[21] have described a well established case, primary in the region of the pineal gland.

showed marked atrophy, tubular fibrosis, and interstitial cell hyperplasia. Bilateral gynecomastia present. Aschheim-Zondek test positive.

In the above indisputable cases the primary sites were mediastinum (2), urinary bladder (2), retroperitoneal (3), and in 1 case either in the hilum of the lung or in the retroperitoneal tissue. In all instances the disease was fatal. The duration of life after onset of symptoms varied from 2 weeks to 15 months (average 5½ months).

Secondary changes in the breasts and testes are not uncommon in cases of choriocarcinoma. The enlargement of the breasts is usually bilateral and may be the first sign associated with such a tumor. The increase in size results from proliferation of ducts and stroma (Fig. 5). Definite gynecomastia was noted in 3 of the above 8 accepted cases, was doubtful in 1, and absent in 4. In the doubtful case [16] a diagnosis of adenofibroma was made on one breast which was removed prior to the patient's death. It seems possible that gynecomastia could have been mistaken for such a tumor.

The testicular changes include atrophy of the entire organ, fibrosis and hyalinization of the seminiferous tubules, and hyperplasia of the interstitial cells of Leydig (Figs. 6 and 7). Such features were noted in 7 of the 8 accepted cases. Similar changes have been observed in primary teratomas of the testes and in many other conditions such as senility, tuberculosis, cryptorchidism, and hermaphroditism. Houghton [18] described similar testicular alterations in a case of well differentiated malignant mediastinal teratoma.

Report of Case *

A white male, 13 years old, was first admitted to Mount Sinai Hospital, Cleveland, Ohio, in January, 1936. For 8 months he had had pain, intermittent in character, in the region of the left nipple. He was well nourished and normally developed. There was diffuse pulsation of the entire upper one-half of the thorax. Tactile fremitus and resonance were diminished over the upper third of the left lung. A tumor was located and removed from the superior mediastinum (Fig. 1). It extended laterally between the lobes of the left lung and was adherent posteriorly to the pericardium. Gross examination of the specimen revealed an irregularly nodular mass which weighed 385 gm. and measured 13 by 12 by 8 cm. Except in one area, 1 cm. in diameter, the mass was completely encapsulated. The bulk of the tumor was solid, with a few irregularly distributed cysts containing gelatinous and grumous material. Microscopic examination revealed it to be a complex teratoma. There were no trophoblastic elements and there was no evidence of malignant change. The following structures were identified: skin, hair, glands (sebaceous, serous, mucous, and salivary), mucous membrane (like that of upper respiratory tract and colon), brain, ganglia, nerves, smooth and skeletal muscle, fibrous connective tissue, fat, cancellous bone, and cartilage.

The patient recovered and was apparently well until July, 1942, 6½ years after

* We are indebted to Dr. S. O. Freedlander for permission to publish this case and to Dr. B. S. Kline for making available the gross specimen, microscopic sections, and photograph (Fig. 1) of the surgical specimen.

the operation. At this time a routine roentgenogram of the chest, taken on examination for entrance into the Navy, revealed a mediastinal tumor with multiple metastases to the lungs. In October, 1942, he was given 30 deep x-ray treatments to the lungs. The metastases increased in size during the treatments. In January, 1943, the patient was admitted to Lakeside Hospital. At this time physical examination revealed an emaciated white male, 21 years old. His temperature was 38°C.; pulse, 105; respirations, 24; blood pressure, 115/65 mm. of Hg. The breasts were increased in size, firm, and nodular. A spherical, firm nodule, 2 cm. in diameter, was present in the subcutaneous tissue of the right upper quadrant of the abdomen. The testes, which were present in the scrotum, were abnormally small. Secondary sex characteristics were well developed.

Laboratory Examination. Urine: trace of albumin, an occasional white blood cell and granular cast. Blood: 16,500 white blood cells, 2.07 million red blood cells, 36 per cent hemoglobin (Sahli), negative Kline exclusion test. Hormone assays; results given in Table I.

Soon after hospitalization the patient's condition was recognized as hopeless. Testosterone propionate (25 mg. per day) was given for 1 week in a futile attempt to depress the growth of the tumor. Because of the insistence of relatives he was given several blood transfusions. On his 11th hospital day the patient developed signs of bronchopneumonia. His temperature, which had varied between 36.7° and 38°C., rose to 39.5°C. Sulfadiazine was given without effect. He died on his 17th hospital day, 7 years after the teratoma was removed from the mediastinum.

Autopsy (no. 8014, performed by Dr. J. C. Sherrick) revealed a circumscribed tumor in the superior mediastinum and metastatic tumors in subcutaneous tissues of anterior abdominal and posterior thoracic walls, left deltoid muscle, lungs, right parietal pleura, diaphragm, liver, kidneys, jejunum, ileum, thoracic and abdominal lymph nodes, and greater omentum. Other important diagnoses were gynecomastia, fibrosis and interstitial cell hyperplasia of testes, and bronchopneumonia.

The mediastinal tumor (Fig. 2) was slightly to the left of the midline. It was spherical in shape, completely encapsulated, and measured 6 cm. in diameter. It was in large part solid but contained scattered cysts filled with translucent, yellow, gelatinous material. Near the periphery there were a few small, soft, hemorrhagic foci. Microscopically its complex nature was indicated by the presence of skin, fat, fibrous connective tissue, smooth muscle, hyaline cartilage, cancellous bone, salivary gland, ganglia, and mucosa, submucosa, and muscularis of bronchus and stomach. In addition, there were cell masses having the structure of a choriocarcinoma. Syncytia and cells of Langhans' type, which normally make up the trophoblastic covering of the chorionic villi, were present (Figs. 3 and 4). Such groups of cells could be located macroscopically by associated hemorrhage and necrosis. The metastatic tumor was similar at all sites. The nodules varied from 1 to 3 cm. in diameter, were sharply circumscribed, and in most instances dark red. Microscopically they contained syncytial and Langhans' cells. The red discoloration was due to recent hemorrhage. Small foci of necrosis were common.

Microscopic examination of the breasts revealed proliferation of ducts and stroma. The ducts were lined with stratified columnar epithelium. The periductal connective tissue was loosely arranged and sparsely infiltrated with lymphocytes (Fig. 5).

In order to exclude a primary testicular tumor, serial sections of the testes were made. The sections were cut at 6 μ and every tenth section was mounted. No tumor or scar was identified. This examination, therefore, excluded any lesion in the testes greater than 0.06 mm. in diameter. Both organs were small, weighing 7 and 6 gm. There was no spermatogenesis. The seminiferous tubules were small and hyalinized. The interstitial cells were markedly increased in numbers (Figs. 6 and 7).

Comment

During life the presence of gynecomastia and small testes suggested to the clinician the possibility of a feminizing tumor. For this reason hormone assays of the urine were done. The results were striking and

Table I

Hormone Assays on 24-Hour Urine Specimens

	Gonadotropin (chorionic type) in mouse units per 24 hrs.	Estrogen in international units per 24 hrs.	17 ketosteroid mg. per 24 hrs.	Pregnanediol mg. per 24 hrs.
Present case	300	750 or more	27	2 or more
Normal male	0	75 or less	25	0.1
Normal nonpregnant female	0	50 to 300 (ovulation)	12	45 (in luteal phase)
Teratoma of testis	Elevated	Not known	Not changed	Not known
Late pregnancy	6000	10,000	Not changed	30 to 40
Choriocarcinoma of uterus	200,000 to 1,000,000	Elevated	Not changed	Not known

probably diagnostic of choriocarcinoma. A comparison of the hormonal findings in this case with those in normal persons and related conditions is given in Table I.

In this case there is no way of determining whether the mediastinal tumor present at autopsy represents the growth of a small portion of the original teratoma left at the operation 7 years prior to death or the growth of another tumor of similar and subsequent origin.

The presence of chorionic gonadotropin in the urine along with increased excretion of estrogens and of pregnanediol is similar to the hormonal alteration which occurs in pregnancy. It may be presumed that these hormones or their precursors were elaborated by the chorionic tissue of the tumor.

Atrophy of the testicular tubules and gynecomastia were undoubtedly due to the large amount of circulating estrogen. A contributory stimulus to the enlargement of the breast may have been added by progesterone.

No exact quantitative method was used to determine the number of Leydig cells in the testes. It was estimated, however, that these cells were increased in number. The examination of a large number of sections of the testes to some extent decreases the error inherent in such an estimation. An actual increase in the number of testicular interstitial cells could be due either to direct stimulation by chorionic gonadotropin or to increased luteinizing hormone secreted by the anterior lobe of the pituitary gland in response to the high level of circulating estrogen. The work of Collins [19] indicates that Leydig cell hyperplasia may occur in cases without carcinoma and in the absence of known excess of luteinizing hormone or estrogen. From his work it is also apparent that hyperplasia of the interstitial testicular cells is associated with atrophy and fibrosis of the tubules but that tubular fibrosis and atrophy may occur without a significant change in the number of Leydig cells.

Summary

Review of the literature discloses only seven well established cases of extragenital choriocarcinoma in males. In the additional case reported in this paper a complex teratoma originating in the thorax of a boy, 13 years old, was removed surgically. Seven years later he died of either a recurrence or an independent teratoma with choriocarcinoma in the tumor and widespread choriocarcinomatous metastases. Gynecomastia, testicular atrophy, and hyperplasia of the interstitial cells of Leydig were associated. Hormonal alterations resembled those of pregnancy.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 154

FIG. 1. Tumor removed from upper mediastinum, 7 years before patient's death. This was a complex benign teratoma. The metric scale which is reproduced is for comparison with Figure 1 only.

FIG. 2. Sections of lungs with mediastinal teratoma attached to medial aspect of upper lobe of left lung. Multiple hemorrhagic metastatic choriocarcinomatous nodules in both lungs.

1

2

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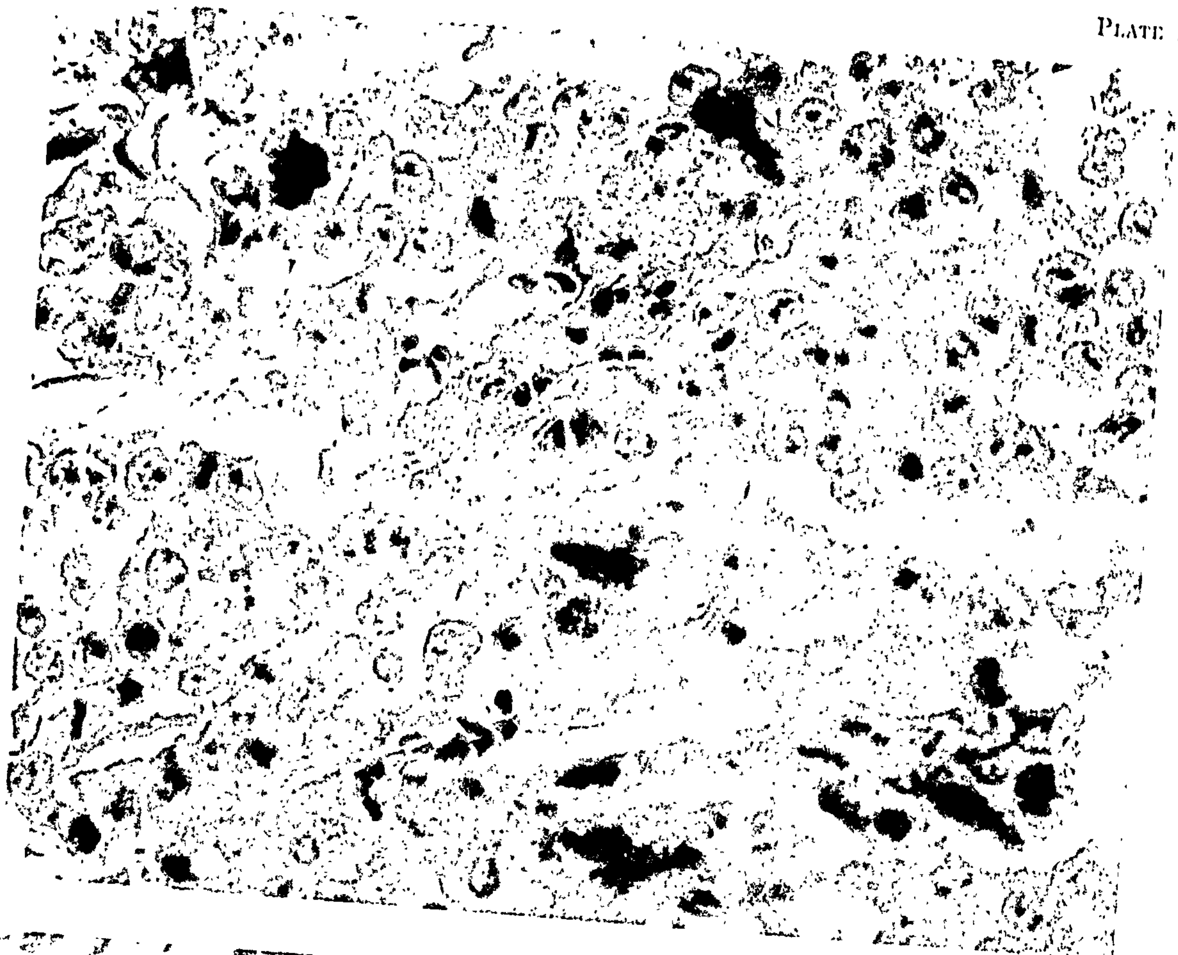
PLATE 155

FIG. 3. Pulmonary metastasis with syncytia, many cells of Langhans' type, and mitotic figures. Hematoxylin and eosin stain. × 538.

FIG. 4. Pulmonary metastasis. Discrete syncytia and groups of Langhans' cells are evident. Hematoxylin and eosin stain. × 582.

3



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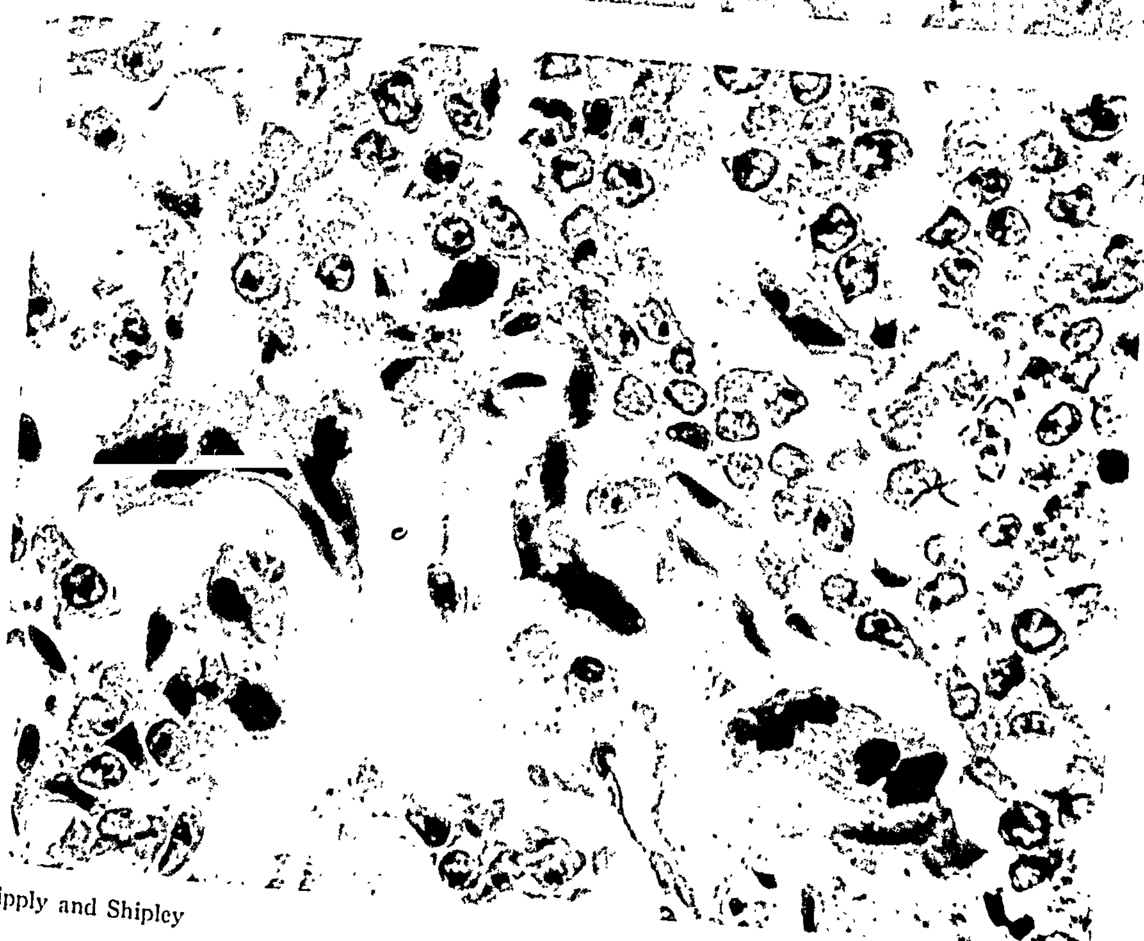

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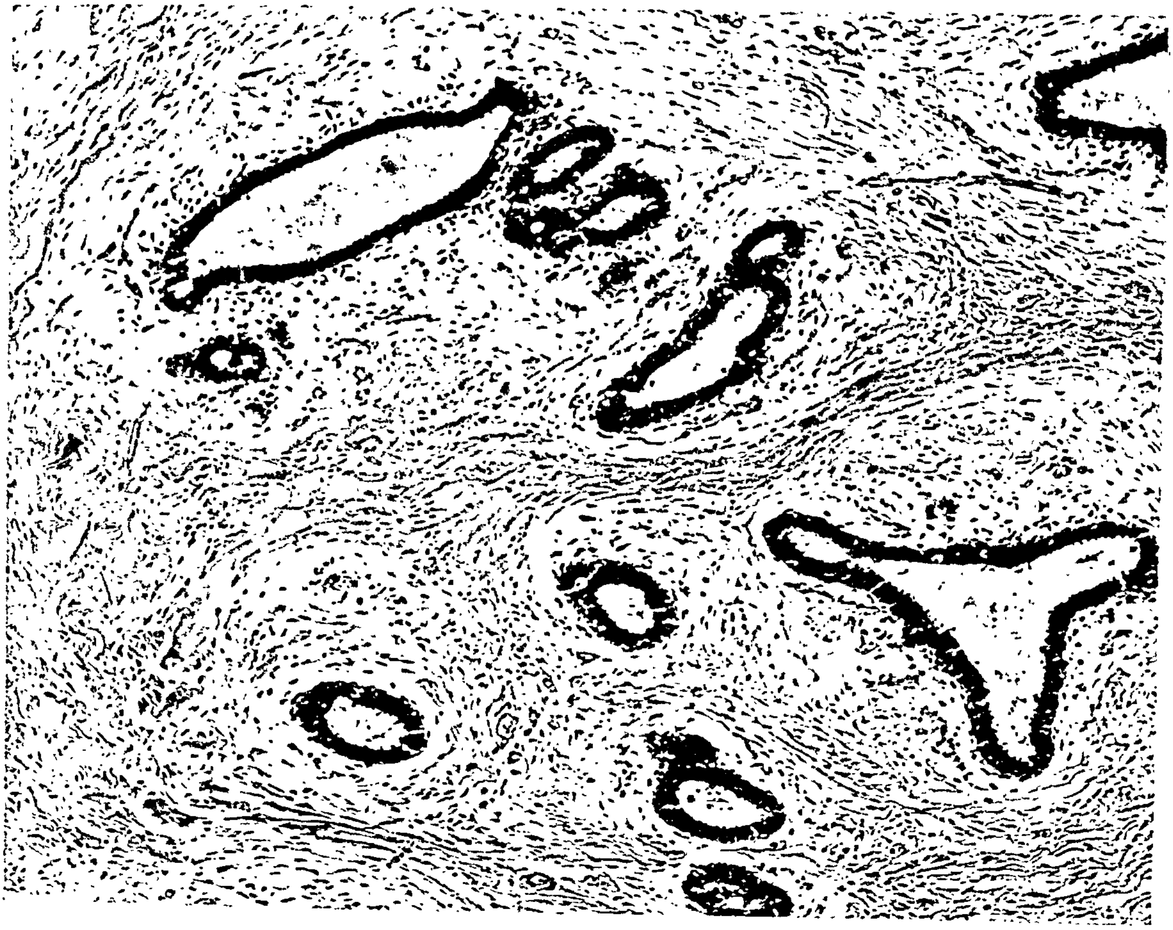
Extragenital Choriocarcinoma in the Male

PLATE 156

FIG. 5. Ducts of breast are increased in number and lined with stratified columnar epithelium. Loose cellular periductal connective tissue is infiltrated with lymphocytes. Hematoxylin and eosin stain. × 94.

FIG. 6. Atrophy and hyalinization of seminiferous tubules and hyperplasia of interstitial cells of testis are evident. Hematoxylin and eosin stain. × 140.

FIG. 7. Marked hyperplasia and pleomorphism of interstitial cells of testis. Hematoxylin and eosin stain. × 140.



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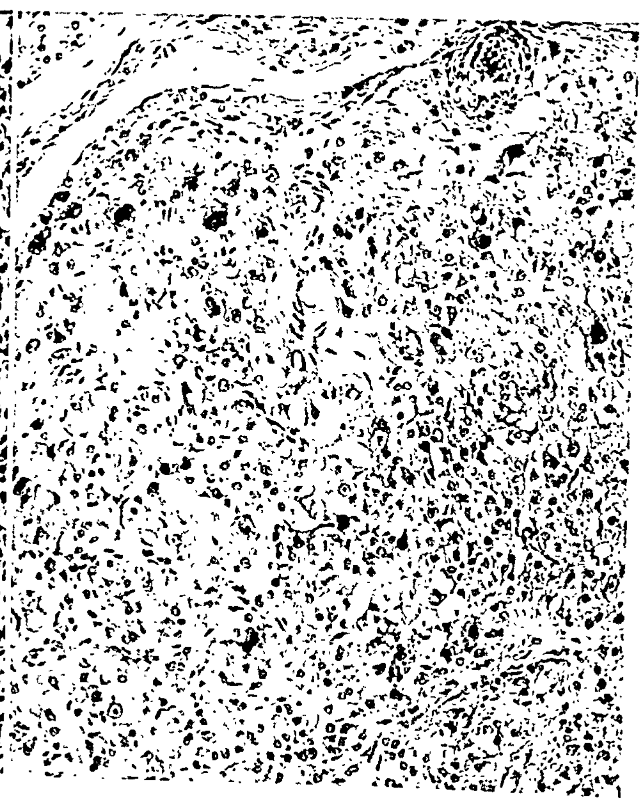

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Extragenital Choriocarcinoma in the Male

STUDIES ON AMEBOID MOTION AND SECRETION OF MOTOR END-PLATES

VI. Pathologic Effects of Traumatic Shock on Motor and Sensory Nerve Endings in Skeletal Muscle of Unanesthetized Rats in the Noble-Collip Drum *

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The morphologic effects of traumatic shock upon the motor and sensory nerve endings in skeletal muscle are unknown. Morphologic evidence has been presented in previous studies [1] which supports the statements that the pleomorphism of the hypolemmal axons of the motor end-plates is due to ameboid motion and that the changes in amount of the granules of Kühne are evidence of different phases of periodic activity of the secretory mechanism of the motor end-plates. Evidence has likewise been presented that there is a metabolic destruction of the motor end-plates by lactic and other acids.[2] There is, furthermore, an acute anatomic breakdown of motor end-plates in hemorrhagic shock.[3] The lesions of the motor end-plates in poliomyelitis,[1] in the experimental injection locally of lactic acid,[2] and in hemorrhagic shock [3] are quite similar. When shock is produced by hemorrhage,[4] trauma,[5] or gravity [6] there is an accumulation of lactic acid and keto-acids in the blood. Gesell and his colleagues [7] have presented important evidence that an accumulation of lactic acid retards the destruction of acetylcholine at the synapses. This local increase of metabolic acids at the neuromuscular apparatus may play a rôle in the alterations of the junction between nerve and skeletal muscle. Shock produced by direct hammer trauma of the living, skin-intact muscle [8] is similar in some respects to the traumatic effect of the Noble-Collip drum on the neuromuscular apparatus. The morphologic effects of shock from any cause upon the neuromuscular apparatus of skeletal muscle have remained an unexplored field. A closer study of the pathology of this region may help to explain the mystery of the onset of shock. Cannon [9] (page xi) in his excellent monograph on shock stated: "....the mystery of the onset of shock has not been definitely cleared away despite a considerable increase in our knowledge of it, and that there still remains

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much work to be done before we shall have elucidated all the factors which play a rôle in its establishment."

and, on page 111:

"An explanation for the development of a low blood pressure, apart from hemorrhage, has not been offered. It is at this point that most diverse opinions arise. Many theories have been offered, often with experimental support, to account for the development of low blood pressure."

Moon[10] in his comprehensive, thought-provoking review of the subject of shock pointed out the significant fact that the pathology of shock was an ignored subject until his own experimental studies. He made the following significant statements (page ix):

"A survey of recorded studies on shock reveals the remarkable fact that in no instance had it been investigated from the standpoint of pathology. Probably this was because it was interpreted entirely as a functional disorder having no morphologic basis. The belief that post-mortem examinations reveal no significant changes following death from shock, had been accepted apparently without question. The problem might have been clarified earlier if the methods of pathology had been combined with those of physiology in the search for pertinent evidence. In this as in other conditions of disease, valid interpretations find corroboration in the accompanying morphologic changes."

In the present state of the lack of knowledge of the pathologic effects of traumatic shock on the secretory mechanism of the motor end-plates, an adequate number of clear-cut photomicrographs is necessary. Concrete evidences of the morphologic changes of the motor end-plates resulting from trauma reveal more specifically than words that there is an ephemeral and tenuous secretory liquid transmitted at the junction between motor nerves and skeletal muscle. By teasing large amounts of muscle, various phases in the pathologic alterations of this secretion are found. By teasing muscle after gold impregnation, the anatomic relationship and the polarity of the secretory mechanism of the neuromuscular apparatus are preserved. The relationships of the epilemmal axon, hypolemmal axon, ramifications of the hypolemmal axon, the discharged granules of Kühne, and the related cross striations of the muscle fiber are revealed in one observation. This anatomic arrangement is obscured by the sectioning method even though the cytologic details of the nuclei and neurofibrils are better revealed by sectioning than by the gold-and-teasing method. Both methods, therefore, should be employed in the study of the neuromuscular apparatus.

A pertinent statement in reference to the gold method, written by Kühne[11] (page 3) in 1887, may be translated as follows:

So much has been written about the use of gold in the staining of muscle and nerve fibers that one has good cause to be brief about it, in particular, since those who have used the method themselves know only too well how little can be learned from numerous descriptions of the procedure and that only individual trial and long experience with the method can lead to sure results. Even the beginner can experiment with making motor nerve endings visible by the gold method, utilizing the action of a reducing medium with which is linked the name of Cohnheim, who

first in histology had success with the gold method; how the gold picture comes out and what structural secrets of nerve endings it hides is still unsatisfactorily known, regardless of the abundance of experimentation with it.

The terminations of nerves at synapses and in muscles and glands have been difficult to study in the past because of technical limitations and more especially because of the unappreciated inconstancy of the structural arrangement related to the different states of the secretory activity stopped by death. Bremer [12] made the following statements:

"Like all nerve endings they [motor endings] are difficult to study because of the unreliability of the staining methods necessary to bring out the fine nerve fibers as distinct from the tissues among which they run. Impregnation methods [silver and gold] and supravital staining with methylene blue are most commonly used, but both require great care in interpretation of results."

The gold-and-teasing method may be easily mastered with persistency and experience. When the objective findings are presented, anyone may make the interpretation.

The purpose of this paper, therefore, is the presentation of direct and conclusive experimental morphologic evidence, in the form of easily verified and clear-cut, untouched photomicrographs, that supports the following theses: (1) that the experimental pleomorphism of the hypolemmal axons of the motor end-plates is the result of normal and abnormal functional ameboid motion; (2) that the experimental variation in the quantity of the granules of the sole plate of Kühne is the structural expression of the differential phases in the secretion of a chemical substance, possibly acetylcholine, from the terminal axons of the motor end-plates; (3) that the gold-staining axonic inclusion masses found within and between skeletal muscle fibers is possibly the morphologic expression of the delayed dissolution of acetylcholine; (4) that traumatic shock produced in unanesthetized rats in the Noble-Collip [13] drum results in the abnormal secretion of axonic substance into the muscle, which secretion, in some instances, leads to a loss of motor innervation at the myoneural junction by exhaustion due to hypersecretion of the nervous liquid; and (5) that the pathologic changes of the muscle fibers in traumatic shock are evidence of the profound underlying changes in the biochemical status of the muscle.

MATERIALS AND METHODS

The gold chloride method previously described,[14] followed by gentle teasing of the motor nerves and muscle fibers, is superior to any other method for our purposes. It reveals the subtle and evanescent neuronal secretion of the motor end-plates in the skeletal muscle fiber as well as the anatomic relationship and polarity of the motor end-plate, epilemmal axon, granules of Kühne, and the cross striations of the muscle fiber. The motor end-plates in the gastrocnemius muscle of the white

rat (*Mus norvegicus*) were stained also by the Bielschowsky method of silver impregnation as modified by Boeke; [15] by the *intra vitam* methylene blue method of Ehrlich as modified by Huber,[16] and by Huber and DeWitt; [17] and by the Ranvier gold chloride method as modified by Wilkinson [18] and by one of us.[14] The spinal cord and brain from traumatized rats were serially sectioned and stained for Nissl substance.

The Bielschowsky silver technic reveals the fine neurofibrillar, reticular nature of the fronds of the axon. The sarcoplasm of the sole plate is clear and unstained by methylene blue. The gold chloride method, however, demonstrates the sole plate (when it is present) to be composed of fine granules. There is a narrow, clear area between the fronds or branches of the terminals and the granular sole plate in some places. In certain expanded plates there is direct continuity between the serrated projections of the terminations of the axon and the dark muscle striae. The ovoid nuclei of the sole plate remain unstained by the gold method and form clear spaces surrounded by dark granules. The periterminal network of Boeke is best seen in expanded nerve plates after staining by the silver method. It forms a foamlike reticulum between that of the nerve terminals in the plate and the periterminal network in the sarcoplasm of the sole plate. In the expanded nerve plates, which appear to be chemical secretory apparatus, there is an apparent finer structural continuity between the terminal nerve plate and the surrounding granular sole plate when stained by the silver method. In the retracted plates this apparent continuity is lost. The line of demarcation between nerve terminals and sarcoplasm is not a constant one. It depends upon the state of functional activity in which life is stopped and the tissue fixed. Although the structural integrity of both muscle and nerve is preserved at the myoneural junction, it appears that some chemical secretory granules pass from the nerve end to the muscle in some rhythmic manner. Are these granules acetylcholine? Evidence is accumulating which supports an affirmative answer.

It may be that the initial use of lemon juice, with its citric acid content, to increase the permeability of the fresh muscle and nerve tissue for gold chloride likewise prevents or retards the dissolution of acetylcholine. For this reason the anatomic demonstration of hyperchromatic gold-impregnated masses in the muscle fiber may be specific for acetylcholine or some related substance discharged from nerve into muscle. Gesell and co-workers [7] have found that increase of acidity by lactic acid retards the destruction of acetylcholine physiologically deposited at the synapses. The other histologic methods with silver, methylene blue, osmic acid, and hematoxylin and eosin, followed by sectioning, do not conserve the normal and abnormal morphology of the neuromuscular apparatus as does the modified gold technic.

One hundred and ten white rats were used in this study and over 2500 slides of teased muscle were obtained from a survey of over 20,000 slides. Experimental traumatic shock without hemorrhage was produced in unanesthetized white rats, average weight 200 gm., in the Noble-Collip [13] drum (Figs. 1 and 2). The underlying principle of the Noble-Collip drum is to traumatize the rat by placing it in a revolving circular drum in which are projections or bumps. These carry the animal up the side during a turn and then drop it, to be picked up by the following projection. The distance the animal falls is such that obvious hemorrhage or fracture of the bone is not produced. The drum has an inside diameter of 18 inches and a depth of 9 inches. The projections are of a blunt triangular shape on cross section with a height of 2 inches and a base of 3 inches. The outside of the drum is closed by a circular, hinged lid in which there is a cellophane window for observation of the animals during the revolution of the drum. The animals used were maintained under standard conditions. The rats which survived were killed at various intervals after traumatization. Post-mortem examinations were made on all animals and our findings agree essentially with those reported by Noble and Collip. They found the most satisfactory drum speed to be 40 r.p.m. In their male rats there was a 100 per cent mortality within an average time of 50 minutes after 700 revolutions. In female rats there was a 75 per cent mortality in the average time of 81 minutes after 700 revolutions. They likewise found that when the rats were subjected to 800 revolutions there was a 100 per cent mortality in the average time of 47 minutes in the male and a 100 per cent mortality in the average time of 39 minutes in the female. The time of death after shock increased as the percentage of mortality and number of revolutions became reduced.

We agree with Noble and Collip [13] that the death or survival of the animal is the most accurate indicator for experimental shock. Blood pressure changes are influenced by anesthesia and hemorrhage. Blood volume measurements are uncertain through the loss of the dye into the tissues. Hemorrhage influences both the red cell counts and hemoglobin determinations. Many of the chemical changes may be interpreted as secondary. Rectal temperatures below 95°F. are frequently found as well as severe hemoconcentration. Rats removed from the drum after 700 or 800 revolutions were either listless or in ante-mortem rigor and appeared ill, but were not unconscious. Respiratory rate was usually increased, slight hemorrhage might be found around the mouth and nose, and in some the incisor teeth were broken. In animals that die soon after removal from the drum the muscles may be either flaccid or in a condition of ante-mortem rigor. Some muscles manifested pronounced fasciculation. The respirations became progressively slowed.

There was considerable variation in the time of appearance of the signs and symptoms of shock, a fact which confirms previous findings of the variable resistance of rats to trauma.

The changes seen in the various organs vary in intensity depending upon the degree of trauma and the time of death after trauma. The muscles in general appear redder and more vascular than normal. There is extreme vascular dilatation, engorgement, and congestion of the mesentery and bowel. Petechial hemorrhages occur quite frequently in the stomach and small and large intestine. The kidneys are usually congested and some specimens of urine contain blood. The spleen is frequently enlarged and congested. The blood is dark-colored and thick in the liver, heart, and lungs. These visceral changes are not very striking in animals that die within 5 to 10 minutes after trauma. Histologically the viscera present changes comparable to those reported by Noble and Collip [13] and by other investigators.

The pathologic effects of hemorrhagic shock [3] on the neuromuscular apparatus in the gastrocnemius muscle of the white rat have been reported. The effects on the motor end-plates of traumatic shock produced by the Noble-Collip drum have not been reported heretofore. The 110 rats that constitute the basis of this report were divided into four series of 25 rats each and one series of 10 used as controls. Series I was composed of those rats that were killed by decapitation immediately after 400 revolutions in the Noble-Collip drum rotating at the rate of 40 r.p.m.; series II, the rats that were killed within 20 minutes, after 800 revolutions; series III, the rats that were killed 30 minutes after completion of 800 revolutions; and series IV, the rats that were killed 40 to 60 minutes after completion of 800 revolutions.

Moon [10] stated that hemorrhage, infection, and anesthesia were complicating variables involved in the different experimental methods previously used to produce direct traumatic shock. Noble and Collip [13] have devised a method in which trauma is applied in a graded, controlled manner, following which a typical picture of shock developed, uncomplicated by hemorrhage, infection, or anesthesia.

Results: Experimental Findings

1. The Pleomorphism of the Normal Motor End-Plate

It was a difficult problem to establish an absolute standard for the structure of the normal motor end-plates. These varied to the point of complete dissolution, in correlation with the elapsed time after death and the degree of activity prior to death. During post-mortem rigidity a rapid disappearance of the motor end-plates occurred in skele-

tal muscle, associated with a considerable local increase in hydrogen ion concentration. This increase was due mainly to a local accumulation of lactic acid, which usually reaches a concentration of from 0.05 to 0.3 per cent in rigor mortis according to the findings of Fletcher and Hopkins.[19] The local increase of acidity by the injection of lactic acid produced rapid dissolution of the motor end-plates.

These findings indicated that the end-plates must be studied as early after death as possible and preferably from muscles excised from animals prior to the cessation of cardiac and respiratory activity. Various anesthetic agents such as ether, chloroform, and pentobarbital sodium modified the structure of the end-plate. It was necessary, therefore, to know in detail the means of death, such as anesthetic agent, bleeding, decapitation, and fatal trauma, in establishing a relative morphologic norm for the motor end-plate. Under light anesthesia (4 mg. pentobarbital sodium per kg. injected intraperitoneally), both gastrocnemii muscles in 10 rats (average weight 200 gm.) were excised 15 minutes after the injection of the anesthetic agent. The muscles were immediately processed by the gold technic.

The length of these relatively normal motor end-plates measured in the long axis of the muscle fibers in the gastrocnemius muscle varied from 20 to 60 μ; the breadth was from 10 to 40 μ and the thickness from 5 to 15 μ. The mean for the length of 1000 relatively normal motor end-plates was 30 μ, and the mean for the width, 22 μ. The mean of the diameter of 1000 muscle fibers was 52 μ. In size and shape these normal motor end-plates, therefore, were highly variable (Fig. 4). There was a quantitative change of the granules of Kühne in the sole plate. They were increased in amount in the relatively retracted end-plates related to muscle relaxation and had an increased staining capacity for gold. In the normally expanded end-plates, associated with muscle contraction, the granules were decreased in amount and more dispersed than in the retracted end-plates. The variations in the size, shape, and number of the branches of the hypolemmal axons of the motor end-plates have been presented previously as evidence of an underlying ameboid motion. The differences in the amount and capacity to take the gold impregnation of the granules of Kühne were concrete evidence of a subtle secretion process that occurred periodically at the neuromuscular apparatus.

The normal retracted axons of the end-plate under high magnification had a rim of Kühne's granules and an intervening light space in some places (Fig. 44). The normal expanded axons of the end-plate, under high magnification, had a diminution or complete absence of the surrounding rim of Kühne's granules (Fig. 46). Although these rela-

tively normal retracted and expanded motor end-plates were obtained from trees of innervation during the early stages after they were subjected to trauma, they were very similar to those obtained from the normal motor nerve tree (Fig. 4). Normal and abnormal motor end-plates were intermingled on the motor nerve trees during the early stages of muscle trauma. There was a progressive pathologic change leading to complete denudation of epilemmal axons of end-plates in the later stages of muscle trauma. This depended upon the intensity and duration of the traumatic stimulus and variation of resistance to trauma of the individual living organism.

2. *The Experimental Production of Axonorrhea of the Motor End-Plates in Traumatic Shock*

The best time to detect axonorrhea, or the enormous outpouring of axonic material into the muscle fiber from motor nerve endings increased in permeability, was between 5 and 15 minutes after the animals had been subjected for 20 minutes to 800 revolutions in the Noble-Collip drum. Large quantities of muscle must be teased in order to detect this phase in the hypersecretion from the motor end-plate. This axonic material, discharged from the motor end-plates, had an intense affinity for gold, taking a more intense impregnation than the cross striations of the muscle fiber. It was found both under the sarcolemma within the muscle fiber, and outside of the sarcolemma between the muscle fibers. This secreted axonic material varied in length from small granules of 0.5 μ to elongated masses of 1000 μ, and in width from 0.5 to 60 μ (Figs. 3, 5, 7, 13, 16, 24 to 27, 39 to 41, 43, and 64 to 68, Kg.). The internal structure of this axonic material was either homogeneous or cross-striated. In some locations the cross striations agreed with the periodicity of those of the muscle fiber. In other locations there was no agreement between these cross striations of the extra-axonic material and those of the muscle fiber.

In certain trees of motor innervation various steps in the descent and discharge of axonic material were followed through the epilemmal axons, motor end-plate, and into the muscle fiber (Figs. 9, 11 to 14, 18 to 29, and 36 to 41). Some of the end-plates had a single island of gold-chromatic material in the center which gave the appearance of the hub of a wheel (Figs. 34 and 45). Other plates were expanded and open in the center (Fig. 35). Some were relatively normal, with retracted hypolemmal axons surrounded by a dense rim of Kühne's granules (Fig. 44), or expanded, with a decrease in the amount of Kühne's granules (Fig. 46). Various steps in the descent of the hyperchromatic gold material through the epilemmal axons to the end-plates were like-

wise clearly evident in various nerves of the same tree of innervation (Figs. 46 to 48). In some places the epilemmal axon was beaded and very little axonic material was found in the hypolemmal axons of the motor end-plate. On the other hand, in neighboring end-plates of the same tree of innervation, large amounts of axonic material were found. This quantitative increase of the gold-chromatic material in the end-plates was detected by the intense impregnation. In other end-plates the beginning of the discharge of the gold-chromatic material was detected extrinsic to the hypolemmal axons of the end-plate. The gold-chromatic material, therefore, was either intra-axonic or extra-axonic. That which was extrinsic to the axons of the end-plate was either in small or large fusiform masses (Figs. 7, 14, 27, 40, 41, and 62 to 68), globules, or droplets (Figs. 5, 6, 9 to 12, 20, 22, 23, 29 to 33, and 36 to 38). The arrangement of the discharged axonic material was unipolar (Figs. 22, 25, and 37), bipolar (Figs. 23, 26, and 36), or multipolar (Figs. 13, 21, 24, and 27).

Within 5 to 15 minutes after the rat had revolved 800 times in the drum some of the end-plates of the motor trees had an increase of the gold-chromatic material as detected by augmented impregnation (Figs. 5 to 11, 16 to 20, 42, and 43). There was a progressive decrease to the point of complete disappearance of the gold-chromatic material in the hypolemmal axons of many end-plates (Figs. 8, and 49 to 57). In some motor trees the gold-chromatic material had disappeared from both the epilemmal and the hypolemmal axons of the motor end-plates (Fig. 58). The process of hypersecretion, therefore, of the axonic material into the muscle produced complete disappearance of the motor end-plates (Figs. 8 and 59), and a gradual depletion of the axonic material in the epilemmal axons in a centripetal direction from the end-plate. In some muscle fibers the liquefaction leading to disappearance of the motor end-plates was indicated by a corresponding degeneration and liquefaction locally of the cross striations of the related muscle fiber (Fig. 8). The epilemmal axons first became beaded, then fragmented, and finally there was complete disappearance of the gold-chromatic material.

The rat in the terminal stage of traumatic shock, decapitated 1 hour after 800 revolutions during 20 minutes in the Noble-Collip drum, had 3,124 end-plates denuded from 3,500 epilemmal axons that were counted in the gastrocnemius muscle (Fig. 8). This denervation of muscle at the myoneural junction was comparable to that produced by experimental poliomyelitis in the monkey, poliomyelitis in human muscles, and by the injection of lactic acid locally in the zone of motor innervation of skeletal muscle in the rat. The number of epilemmal

axons denuded of end-plates or possessing pathologic end-plates varied in different fibers within the same muscle as well as in different muscles in the same rat. The variable time factor of denudation of the nerve fibers of their end-plates was, therefore, important: the longer the time after muscle trauma the greater the quantity of end-plates that underwent complete disappearance. There was individual variation of the rats in manifesting this deviation from normal.

The rats of series I that were killed immediately after 400 revolutions in the drum at the rate of 40 r.p.m. had 89 end-plates in a state of extensive axonorrhea in a differential count of 3,156 end-plates in gastrocnemius muscle taken at random. There were individual variations in the animals. In two animals there was no extensive axonorrhea (abnormal discharge from the permeable end-plates) and in one animal there were 10 plates of a tree with 60 endings manifesting extensive discharge of the gold-chromatic material in one of the gastrocnemius muscles. Many end-plates had an increased intensity of gold impregnation (hyperchrysophilia).

The rats of series II that were killed within 20 minutes after 800 revolutions in the drum rotating at the rate of 40 r.p.m. had 267 end-plates in a state of extensive axonorrhea in a differential count of 3,097 end-plates counted in gastrocnemius muscle taken at random. Again there was individual variation in the animals. In one muscle there were 5 motor end-plates manifesting extensive discharge, and in another there were 20 of a tree with 45 endings in a state of extensive axonorrhea. Many end-plates had a decreased intensity of gold impregnation (hypochrysophilia).

The rats of series III and IV that were killed 30 minutes and 40 to 60 minutes, respectively, after 800 revolutions in the drum rotating at the rate of 40 r.p.m. had the majority of the end-plates in various degrees of liquefaction. Extra-axonic gold-impregnated masses (*neurosomes*), discharged from the end-plates into the muscle fibers, were rarely found. The usual appearance of the motor innervation was one in which the epilemmal axons of the motor trees were almost completely denuded of the motor end-plates (Figs. 8 and 59). There were 3,124 end-plates denuded from 3,500 epilemmal axons counted in muscle taken at random and excised 1 hour after rotation in the drum.

3. *Effect of Trauma on Muscle Fibers and Blood Vessels*

Dependent upon the time of observation after muscle trauma and individual variations, some rats were flaccid and others rigid while respiratory and cardiac activities were still going on. Certain localized groups of muscles manifested fasciculation and others periodic con-

vulsive movements. The functional condition of the muscles, therefore, was pathologic and highly irregular in traumatic shock. In the final stages of irreversible shock stimulation of the sciatic nerve failed in many instances to cause a response in the gastrocnemius muscle and in certain other muscles. From the motor innervation in the late stages of shock there was almost complete disappearance of the end-plates (Figs. 8 and 59). Certain other muscles in the end stage of shock were in a greatly reduced state of irritability or had lost completely the capacity to respond to direct electric or mechanical stimulation. Many muscle fibers whose irritability was reduced or lost had so-called Zenker's waxy and granular degeneration (Figs. 8, and 59 to 62). There was a correlation between flaccid muscular paralysis and peripheral nervous exhaustion of the structure at the myoneural junction. Some of the muscle fibers had a series of fusiform structures that were intensely impregnated with gold (Figs. 15 and 61). There were irregularities in arrangement and loss of the cross striations, increased visibility of pyknotic nuclei, and perivascular infiltration of leukocytes (Figs. 15, and 59 to 61). There was a disappearance of the differential fiber types in both a localized and generalized manner. The sarcoplasmic substance in some muscle fibers was increased and in others decreased. Some of the muscle fibers were swollen. In other locations the muscles grossly not only appeared more red than normally but upon microscopic observation the intramuscular capillaries and venules were dilated and contained agglutinated red blood cells accompanied by perivascular edema. In other places these vessels were collapsed and devoid of cellular constituents.

4. *Effect of Trauma on the Proprioceptive Sensory Muscle-Spindles*

There are two to ten intrafusal muscle fibers in each sensory spindle. Each intrafusal muscle fiber maintains connection with two types of nerve fibers: (1) with the large sensory medullated nerve or nerves which arborize in spirals, dendritic branchings, and rings in the encapsulated enlargement, and which conduct proprioceptive afferent impulses upon stretching and increasing the tension of the muscle (Figs. 69 to 71, M.S.); and (2) with the motor medullated nerves which conduct efferent centrifugal impulses by virtue of which the intrafusal muscle fibers of the sensory spindle are capable of normal and abnormal contraction like the other ordinary extrafusal muscle fibers.

In some of the muscle spindles the terminal nerve organ was fragmented by trauma and in other locations the nerve terminals were intact. The most evident pathologic finding was periodic dilatation or beading (Fig. 69, D) of the axons which conduct the sensory impulses

away from the spindle. This moniliform pattern of the sensory axons was apparently morphologic evidence of hyperactivity of the sensory muscle spindle due to trauma. The tendon spindle (Fig. 72, T.S.) gave slight evidence of fragmentation of the end-organ which terminated in globular enlargements of the axons and slight beading of the afferent conduction axon which indicated overactivity.

5. The Effect of Trauma on the Motor Cell Bodies in the Ventral Horn of the Spinal Cord

Under the conditions of the experiment of a short time in the Noble-Collip drum and subsequent death, the anatomic changes of the ventral horn cells in the cord were not so advanced as those demonstrated in the motor end-plates in the muscle of the same animal. During the early stage of trauma many of the cell bodies manifested chromatolysis of the Nissl substance in the cytoplasm. In the late stage of traumatic shock there was slight shrinkage of many motor cell bodies and beginning vacuolization of the cytoplasm. The nucleolus was hyperchromatic but the rest of the chromatic material and the cytoplasm had disappeared from many motor cells. No cell was found in a stage of complete cytolysis, probably because the elapsed time after trauma was too short. Many of the cells were hyperchromatic in animals of series I killed immediately after 400 revolutions in the drum rotating at the rate of 40 r.p.m. On the other hand, the majority of the cells were hypochromatic in the cords of animals killed 40 minutes to 1 hour after 800 revolutions.

Discussion

The pathologic effects of traumatic shock on the neuromuscular apparatus in voluntary muscle amplify the normal neuronal secretion to the point of complete depletion of the terminations of the peripheral nervous motor mechanism and the production, in places, of granular degeneration of the muscle substance. The process of elaboration and discharge of the abnormal neuronal secretion is recognized by changes in the form and contents of the motor end-plates. Under normal conditions this granular secretion is ephemeral and tenuous; the quantitative changes of the granules of Kühne are evidence of it. During the early stages of the abnormal conditions there appear to be both an increase in the amount of the neuronal secretion and a delay in its dispersal and disappearance. During muscle traumatization, discharge of the nervous secretion appears to proceed more rapidly than its elaboration. The process involves a certain polarity of the motor end-plates. The basal pole is directed toward the sarcolemma and receives, through the epilemmal axon, the supply of nutritive material which is

elaborated in the ventral horn cells and along the course of medullated nerves. The secretion is passed out from the opposite or free pole of the ramifications of the hypolemmal axons into the myoplasm. The abnormal extra-axonic secretion accumulates as discrete granules or as homogeneous or striated masses of these granules, which form streamers of various sizes both within and without the muscle fiber.

This polarity of the secretory mechanism of the neuromuscular apparatus is best studied, therefore, in its entirety, in teased preparations impregnated with gold, and not in the ordinary sectioned specimens. When such gold-impregnated, teased specimens from traumatized muscles are examined microscopically, the nuclei of the granular sole plate of Kühne are seen to be either vesicular with distinct chromatin granules, or pyknotic. The morphologic changes in the motor end-plates may simulate those of a holocrine, apocrine, or merocrine gland. Under certain experimental conditions of overstimulation by physical or chemical means the hypolemmal axons of some motor end-plates disintegrate en masse into the secretory granules, comparable to the morphologic changes of a sebaceous gland cell, a holocrine type of gland. We have designated as "axonorrhea" the condition of abnormal outpouring of the axonic material in elongated streamers from the motor end-plates into the myoplasm. The morphologic changes in the nerve endings and muscle fiber are substantial evidence of underlying biochemical alterations in the neuromuscular apparatus. There is morphologic evidence of the chemical theories of the transmission of the nerve impulse proposed by DuBois-Reymond,[20] Langley,[21] Loewi,[22] Dale and Feldberg,[23] Cannon,[24] Gesell, Brassfield, Hansen, and Mason,[25] and others. These pathologic findings in the nerve endings and muscle fibers in voluntary muscle may be involved in the onset of traumatic shock and, furthermore, may be the structural expressions of the initial elaboration of toxic substances in voluntary muscle.

It is interesting to note that during and subsequent to the Civil War in the United States there were theories concerning shock which involved the idea that there was an exhaustion of some subtle nervous fluid. This conception had been discarded until recently because of the lack of physiologic and especially morphologic evidence. In the monograph on shock by Moon [10] (page 95), the following appears:

"The earlier hypotheses were entirely conjectural. Foreign substances in wounds, coagulation and other changes in the blood were proposed as causes. The writings abound in such hypothetical concepts as 'draining of vital fluid,' 'loss of animal and organic powers,' 'destruction of the great nervous power,' 'complete depression of all vital functions,' 'commotio cerebri,' and the like. A detailed review of these theories is not profitable. One instance may be cited as reflecting the character of medical thought within this period. Samuel D. Gross (1872) wrote: 'Shock is a depression of the vital powers, induced suddenly by external injury,

and essentially dependent on loss of innervation. It bears the same relation to the nervous system as syncope to the vascular. In the one case the result is caused by a diminution of the nervous fluid, in the other by a diminution of the blood; in both the consequence is more or less prostration. ... The blood has long been known by physiologists as the vital fluid so necessary to the well being of the system. But it is certainly not the only fluid entitled to this distinction; the nervous fluid is both more subtle and more important as a life preserver. When blood flows away in a mighty and overwhelming torrent... life is destroyed by the excessive sanguinous drainage. But in shock the same effect may happen, and yet the body be literally surcharged with blood, not a single drop, perhaps, having been spilled in the accident causing the fatal result. Thus of the two fluids the nervous is the more important, because the more essential to life; and its disturbance is therefore a more frequent cause of death.' "

Gross based his hypothetical concepts of "loss of innervation" and "diminution of nervous fluid" upon clinical observation. He was nearer the truth than most morphologists have realized up to the present time. The same applies to the prophetic and intuitive statement, "is it that conduction checked somewhere causes at that spot destruction from dangerous accumulations of nerve force?", by that acute observer and clinician, S. Weir Mitchell,[26] in 1872 (pages 142–144), more fully expressed as follows:

"Shock, then, is reflex disturbance, or, in some cases, paralysis of centres. Why in one case the cerebrum should suffer, in another the heart, and in a third the motor centres of the leg or arm, is as yet inscrutable. A ball crushes a nerve, and the tremendous shock instantly propagated to the spine falls ruinously upon some one of the numerous ganglia through which it travels. Is this because it finds a weak point, or is it that conduction checked somewhere causes at that spot destruction from dangerous accumulations of nerve force? . . . .

"These, also, I prefer to explain by presuming that the shock had suddenly exhausted some ganglionic centre, and thus palsied its related muscles. This view was set forth by us in March, 1864, and more elaborately, though not more distinctly, by Jaccoud, in December of the same year. We then remarked that 'either the shock of a wound causes paralysis of vaso-motor nerves and sequent congestion, with secondary alterations, or that it destroys directly the vital power of a centre. Now, there is no reason why, if shock be competent to destroy vitality in vaso-motor centres or nerves, it should be incompetent so to affect the centres of motion or sensation.' Indeed, it appears incomprehensible that any vasal spasm and consequent relaxation could be competent to instantly and permanently paralyze a whole limb, while sudden deaths from shock seem also explicable in no other way than by absolute exhaustion of nerve force in some vital centre."

If the term "vital centre" be replaced by the one "motor end-plates," Mitchell's speculation has anatomic evidence to support its validity. Mitchell,[27] in 1866 (page 355), came to the conclusion that muscle paralysis due to shock was based on nervous exhaustion:

"If I were now to sum up the probabilities in the way of causation of palsies peripherally induced, I should be disposed to refer some cases to exhaustion from too constant or excessive exercise of normal functions, and others to irritation from disease or injury, and to consequent exhaustion of the centres; while, as regards the intervention of vascular agency, I should reject the idea of prolonged vasal spasm, and consider it possible that in some instances over-excitation might

result in dilatation of the vessels, in which case some material lesion would surely follow, if the condition in question were of long continuance."

There is abundant morphologic evidence that traumatized muscle has demonstrable pathologic changes in both the motor end-plates and muscle fibers. These alterations may be the structural expression of profound biochemical changes in the neuromuscular apparatus that may be involved, likewise, in the production of abnormal amounts of certain toxic metabolites. Green and Bielschowsky [28] claimed that adenosine triphosphate may be this toxic substance. In the following statements (page 197) Noble and Collip [13] supported the hypothesis that a toxic substance is produced in the tissues due to trauma:

"The findings which have been obtained up to the present are most readily interpreted by the hypothesis which has been advanced by many that a toxic substance is produced in the tissues due to trauma. If this is the case it would explain why some animals may die immediately following trauma and why, once this type of trauma ceases, the production of a toxic substance stops. In the animals where death follows almost at once the typical shock picture may be only partially developed and haemoconcentration present only to a slight degree. These features must therefore be secondary to the liberation of the toxic substance. It would appear that if enough of the toxic substance enters the blood stream it produces death rapidly, possibly by affecting the heart or brain. In an animal receiving trauma for a short period the peripheral circulation is probably relatively good and therefore the toxic substance may be rapidly removed from the damaged tissue to the blood stream. On the other hand, in an animal subjected to trauma over a prolonged period the circulation may be poor and the toxic product released slowly causing death at a later interval. Somewhat similar conditions may influence the concentration of the blood. In animals dying rapidly little haemoconcentration may be found. However, in the presence of traumatized tissues, over a longer period, marked loss of plasma from the blood may occur. It would appear that if the toxic substance produced remains in the tissues, as when the peripheral circulation is poor, the loss of fluid locally is marked. In such cases the death of the animal is related to a direct toxic effect plus the result of haemoconcentration. Further, when haemoconcentration is advanced the oxygen supply to the peripheral tissues is deficient. Such anoxaemia may lead to a further local increase in the production of the toxic substance. Such an argument would correlate the type of shock caused by methods employing temporary occlusion of the blood supply to some part of the body with that following direct trauma, since the end result would be the liberation of the same toxic product. The above theory for the aetiology of the development of shock, which has been evolved from the experiments described and others to be published, is similar in some ways to that of Moon. However, in the theory outlined the peripheral tissues are suggested as the site where some fundamental chemical alteration occurs following trauma or ischaemia, and this results in the production of a toxic substance. This substance may act locally, resulting in the attraction of plasma with haemoconcentration (which in turn causes secondary general ischaemia and additional liberation of the toxic material), or systemically, possibly affecting the heart and brain, and even producing rapid death. Whether the substance acts locally or enters the systemic system may in itself be determined or influenced by the state of the cardiovascular system and peripheral circulation. In such a plan the capillaries would have a passive rôle and be affected secondarily rather than be the primary site of the damage responsible for the development of the shock picture, as outlined by Moon.

"If this explanation is correct the types of shock, as seen both experimentally

and clinically, might be expected to show marked variations. At the one extreme would be cases where death followed trauma very rapidly, and at the other those which ran a protracted course and in which increasing haemoconcentration was a prominent finding. Therapeutic procedures which tend to alleviate increased blood concentration might be expected, therefore, to be beneficial in the latter type of case only."

Possibly when the pathologic mechanism of shock due to trauma, hyperthermia, hemorrhage, or infection is completely known the voluntary neuromuscular factor may be found closely related to the production of toxins (Moon,[10] Dale,[29] Cannon,[30] Bayliss [31]) and to local fluid loss from capillaries (Blalock,[32] Harkins,[33] Freeman, Freedman, and Miller [34]).

The concept of an axonal secretion of the motor end-plates discharged into the muscle fibers requires a reconsideration of the physical nature of the conduction medium of motor nerves in skeletal muscle. The physical nature of the motor axis cylinder and the means by which the nerve impulse is conducted and discharged in muscle are unknown. After Remak [35] discovered neurofibrils in the living nerve fibers of the crayfish, Schultze [36] presented the hypothesis that the neurofibrils were the specific wire-like conductors of the nerve impulse. What went up or down the neurofibrils to constitute a nerve impulse was unknown.

The prevalent opinion expressed in most textbooks of histology is that the neurofibrils are the specific core electroconductors of the neural impulse. Neurofibrils were found by de Rényi [37] in living nerve fibers of invertebrates. Parker [38] stated that the neurofibrils control the metabolic trophic activities of the neuron, whereas Verworn,[39] von Lenhossék,[40] Koltzoff,[41] Goldschmidt,[42] and von Szüts [43] held that the neurofibrils in the axon are supporting structures for the semifluid neuroplasm.

The theory that the conduction medium of the nerve impulse is a liquid is supported by the observations of Jenkins and Carlson,[44] Carlson,[45] Meek and Leaper,[46] Lapicque and Legendre,[47] Erlanger,[48] Erlanger and Gasser,[49] Gasser and Erlanger,[50] Young,[51] Gerard,[52] and Weiss.[53]

In our own experimental studies [54] evidence was produced that supported the following statements (page 428):

"The physical state of the axis-cylinders in the central and peripheral nervous systems is that of a colloidal liquid. The experimental production of acute traumatic syringomyelia with multiple cavitations of the axis-cylinders of the spinal cord is dependent on the liquid transmission of pressure pulses from the high to the low pressure area by the sudden crushing blow on the central nervous system. The excessive lateral expansions with 'blow outs' of the axis-cylinders by the pressure of mechanical trauma or strychnine stimulation are the result of the liquid transmission of compression. When a nerve is sectioned and stimulated by the faradic current, liquid droplets of the axis-cylinders are expressed from the cut end by the compression pulses of the electric current. The transmission of the

nerve impulse tends to produce multiple lateral expansions of the liquid axis-cylinders which conduct the pressure pulses."

The nerve impulse appears to be transmitted through the entire cross-sectional area of the axis cylinder, which may vary in a reversible manner either partially or completely from a liquid to a semiliquid state. In view of the objective evidence herein presented, neurofibrils can hardly be thought to remain the specific structural conductors of nerve action. The neurofibrils ought rather to be considered as longitudinally condensed parts of the colloidal liquid of the axon drawn out into a microcapillary filament by the tension of relative growth and the streamline effect of metabolic activity and axonic flow. The neurofibrils are inconstant structures; they vary with the chemical condition and physical state and shape of the axis cylinder; they are the physical resultants of longitudinal tension and lateral compression in an extended protoplasmic strand. Comparable physical and chemical conditions form myofibrils in muscle.

Jackson,[55] in his study of visual fatigue, concluded with the following pertinent statements in reference to the alterations in the neuromuscular apparatus in fatigue.

"The number of points at which fatigue might occur so as to lower activity or cause sensations of which we are conscious, are thus enumerated by Herrick,[56] who bases them on the summary of Stiles: [57] (1) fatigue of muscle fibres, (2) fatigue of the junction of the motor nerve with the muscle fibre at the motor end-plate, (3) fatigue of the nerve-fibres, (4) fatigue of the motor nerve cells, (5) of the synapses between the nerve cells, (6) fatigue of the sense organs and afferent apparatus, (7) fatigue of the centers of voluntary control.

"Physiologic experiment shows that the nerve fibres are capable of conducting impulses after the neuro-muscular apparatus has become exhausted and fails to respond to stimulus; and that muscle fibres cease to respond to impulses coming thru the nerve trunk, and yet contract well under direct stimulation. This shows that neither the muscles or the nerve fibre is exhausted.

"Stiles [57] thinks the junction of nerve and muscle is especially likely to give out under continued stimulation. He says: 'One is tempted to draw a comparison between the end-plate and the safety fuse such as is used in connection with an electric fixture. The fuse is intended to be destroyed under conditions which might otherwise threaten damage to more valuable portions of the system. It is readily renewed. So we may think of the end-plate as something easily impaired by use, but also easy to repair. It is better that wear and tear should fall upon this structure than upon the more highly organized protoplasm of nerve cells or muscle fibres.' "

These concepts are supported by the morphologic findings reported in this paper. In fact, before our attention was drawn to the similar analogy previously made by Stiles,[57] we had designated the motor end-plates as chemical safety fuses, basing our designation on facts observed in our lactic acid series of experiments.

The variable time factor involved in the production of the localized and irregular morphologic changes of axonorrhea, as well as the final

disappearance of the motor end-plates in certain parts of different muscles, may account for the conflicting reports on the pathologic function of the neuromuscular apparatus in shock patients quoted by Cannon [9] (pages 1, 2, 3, and 119 respectively):

"*If the limbs are lifted and then let go, they immediately fall as if dead.* ...On being thus questioned he complains of cold, faintness, and deadness of the extremities." (Fischer.[58])

"The muscles are not paralyzed anywhere, but the patient seems disinclined to make any muscular effort." (Warren.[59])

"Thus Mitchell, Morehouse, and Keen,[60] in their report to the Surgeon General of the United States Army in 1864, described cases occurring after wounds of grave nature, in which 'the patient, immediately after being wounded, suffered from a state of depression which continued and which was marked by great weakness, feeble circulation, pallor, etc."

"Cowell [61] *reported no reduction of the strength of grip in shock and he* observed one wounded man, pulseless and showing a systolic pressure of approximately 30 mm. Hg, who was so vigorous in his movements that two orderlies were required to hold him on the stretcher."

Observations by Brown-Séquard [62] demonstrated that the intravascular injection of alkaline solutions in animals and of defibrinated blood in a decapitated criminal removed rigor and re-established muscle irritability; by Wedensky,[63] that indirect overstimulation of muscle through its nerve supply led to inhibition of transmission of the nerve impulse at the motor end-plates; by Wells,[64] that lactic acid caused Zenker's waxy degeneration of muscle; by Norris,[65] that the time of onset of rigor was influenced by the intact peripheral nerves; by Matthews,[66] that occlusion of circulation and muscle activity produced pain and "explosive" changes in the electric potential of the hyperexcitable sensory spindles; by Gesell and his co-workers,[7] that increased hydrogen ion concentration in muscle resulted in the discharge of augmented amounts of acetylcholine at the motor end-plates; and by Rosenbohm,[67] that nerve cell proteolysis was produced by lactic acid.

Morphologic evidence, therefore, is being accumulated that supports the conclusion that normal metabolism maintains, and abnormal metabolism alters, the structure of the motor end-plates in skeletal muscle in health and disease. There is, furthermore, morphologic evidence, clearly demonstrable by the methods of experimental pathology, that the motor end-plates are microscopic endocrine glands that pour their secretion directly into and between the muscle fibers. This supports the chemical theory of impulse transmission from nerve to muscle in the maintenance of normal muscle tonus, contraction, relaxation, and heat production. The hypersecretion of axonal substance into certain pathologic muscle fibers and the denervation at the myoneural junction in shock may be the abnormal changes that underlie the hyperexcitability, spasm, changes in heat production, and flaccid palsy caused by

altered muscle metabolism. These liberate augmented amounts of toxic metabolites under conditions of anoxia associated with overstimulation resulting in structural nervous exhaustion of the neuromuscular apparatus and acute muscular degeneration in localizing areas. A microscopic survey of entire muscles must be made, therefore, before a pathologist is warranted to express an opinion whether muscles are normal or abnormal in either shock or poliomyelitis. There is no short cut by inspection of a few sections of muscle tissue.

Human and experimental poliomyelitis produces alterations of the neuromuscular apparatus very similar to those resulting from the local injection experimentally of lactic acid and to those of shock (hemorrhagic, thermal, chemical, infectious, and traumatic). Whether the changes of the motor end-plates in poliomyelitis are primary or secondary is still undetermined. There is no doubt that the changes produced locally by the injection of lactic acid in the muscle are primary. In the early stages of shock there is considerably greater alteration detected at the myoneural junction than in the cell bodies of the spinal cord. In the late stages of shock the ventral horn cells of the spinal cord likewise show pathologic changes but not to the same degree of dissolution as is found in the motor end-plates.

These changes of the motor end-plates, therefore, are not peculiar to shock nor characteristic for it alone; they are essentially fundamental and capable of general application in many disease processes. The changes may be regarded as the manifestations of functional activity carried beyond normal limits to its termination in fatigue, spasm, flaccid palsy, and structural exhaustion. The sequence of these structural changes is: (1) an increase of the gold-chromatic material (hyperchrysophilia) in the motor end-plate; (2) axonorrhea or the discharge of abnormal quantities of the hyperchrysophilous material from motor end-plates increased in permeability; (3) a progressive decrease to the point of complete disappearance of the gold-chromatic material (hypochrysophilia) in both the hypolemmal and epilemmal axons; and (4) the evanescent appearance of extra-axonic gold-chromatic material in the muscle fibers.

The quantitative increase of the gold-chromatic material in the axons in shock is absolute in comparison to normal changes in the motor end-plates. The actual increase in the gold-chromatic material results from an augmentation of the axonic substance in the motor end-plates which finally are increased in permeability. Under conditions of shock the ordinary granules of Kühne discharged into the muscle fuse into larger masses. These masses of gold chromatic substances are found close to, as well as at a considerable distance away from, the motor end-plates.

These facts are morphologic evidence which supports the conclusion that the motor end-plates are microscopic glands of internal secretion. The essence of this secretory process in shock is the continued overproduction of gold-chromatic material followed by its dissolution to the point of complete structural disappearance of the motor end-plates in skeletal muscle. These morphologic changes may be produced suddenly and with explosive violence or they may be gradual in onset dependent upon the intensity and duration of the abnormal shock stimulus to, and the resistance of, the living organism, especially the living nervous, muscular and vascular systems.

Summary

1. Whole-mount specimens of skeletal muscle and motor end-plates were studied by the gold-and-teasing method under low and high powers of the microscope in the gastrocnemius muscles from 100 unanesthetized white rats traumatized in the Noble-Collip drum. The changes of the motor end-plates in the 200 gastrocnemius muscles of the traumatized animals were compared with 20 relatively normal control muscles excised from 10 animals 15 minutes after the intraperitoneal injection of pentobarbital sodium.

2. There was a sequence of overlapping changes produced by trauma on the neuromuscular apparatus:

(a) The first effect of traumatic shock was hyperchrysophilia (increased intensity of gold impregnation) of the motor end-plates.

(b) The second effect of traumatic shock was an axonorrhea (abnormal discharge from the permeable end-plates) of hyperchrysophilous axonic material into and among the muscle fibers.

(c) The third effect of traumatic shock was a progressive hypochrysophilia leading to disappearance of the motor end-plates due to structural exhaustion. The increased permeability of the motor end-plates produced an abnormal discharge of the chrysophilous material of the axons into the muscle to the point of complete depletion of the axonic structure of the motor end-plates.

3. The extra-axonic material secreted from the motor end-plates into the muscle is an ephemeral and tenuous liquid that forms masses in the muscle fiber, which masses were either homogeneous or cross-striated. This secreted nervous material was found in greatest abundance within 20 minutes after the rats had made 800 revolutions in the Noble-Collip drum rotating at the rate of 40 revolutions per minute. This chrysophilous material secreted by the axons of the motor end-plates disappeared from the myoplasm of the muscle fiber within 1 hour after the rats made 800 revolutions in the drum.

4. The increased quantity of extra-axonic material discharged from the motor end-plates was accompanied in some muscle fibers by an acute granulation, liquefaction, and hyaline degeneration of the myoplasm with loss of the cross striations. Large quantities of the gold-impregnated muscle must be surveyed before a reliable opinion may be rendered regarding the presence or absence of pathologic changes in a specific muscle.

5. It is suggested that overstimulation due to overactivity and trauma of the white rats in the Noble-Collip drum produces increased secretion of the axonic liquid of the motor end-plates into muscle. This results in acute degeneration of some of the muscle fibers. It had been demonstrated previously that increasing the quantity of lactic acid in the muscle destroyed the motor end-plates. Overactivity, likewise, increases the quantity of metabolites, including lactic acid, in the muscle, and produces localized dilatation of the small blood vessels, and causes local perivascular edema. Thus, localized muscle anoxia is produced. Two factors are at work in the structural exhaustion of the motor end-plates: (1) overstimulation of the nervous system, and (2) localized anoxia of the muscle fibers and accumulation of lactic acid, keto-acid metabolites, and other products of anerobic metabolism.

6. The morphologic depletion of the motor end-plates and acute degeneration of certain skeletal muscle fibers are structural expressions of a profound alteration in the underlying biochemical processes of the neuromuscular apparatus. The neuromuscular junction may be one site where toxins are produced. The pathologic changes which occur at the neuromuscular apparatus may be one of the factors in the trigger mechanism which initiates traumatic shock. This initiation may be sudden or slow depending upon the strength and duration of the stimulus, the resistance of the living organism, and the degree of pathologic change in the neuromuscular apparatus. In irreversible shock with flaccid paralysis there is denervation of the muscle at the myoneural junction. The pathologic state of the muscle in shocked rats is highly variable: ante-mortem rigidity, continuous or periodic spasm, fasciculation, or flaccid paralysis may occur. Most of the motor end-plates had disappeared from the tree of innervation in those muscles that failed to respond to both direct and indirect electric or mechanical stimulation. There was a correlation, therefore, between flaccid muscular paralysis and peripheral nervous exhaustion of the structure at the myoneural junction. In some animals the muscles failed to respond to indirect, but did respond to direct electric stimulation. Under such conditions the motor end-plates were either abnormally retracted into ball-like structures or were undergoing acute degeneration.

These facts are morphologic evidence which supports the conclusion that the motor end-plates are microscopic glands of internal secretion. The essence of this secretory process in shock is the continued overproduction of gold-chromatic material followed by its dissolution to the point of complete structural disappearance of the motor end-plates in skeletal muscle. These morphologic changes may be produced suddenly and with explosive violence or they may be gradual in onset dependent upon the intensity and duration of the abnormal shock stimulus to, and the resistance of, the living organism, especially the living nervous, muscular and vascular systems.

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7. Conclusive objective morphologic evidence, confirming our previous studies, is presented to sustain the theory that the motor end-plates are microscopic glands of internal secretion. This anatomic fact confirms the physiologic and chemical findings supporting the chemical theory of the transmission of the nerve impulse.

8. The degree of pathologic change in the motor end-plates is greater than that of the cell bodies of the spinal cord in rats shocked in the Noble-Collip drum.

9. The sensory axons of the muscle and tendon spindles have dilatations indicative of overstimulation.

10. The anatomic changes in the neuromuscular apparatus produced by traumatic, thermal, and hemorrhagic shock are similar to those found in experimental and human poliomyelitis and in certain other diseases in man.

11. Because of the fact that the motor end-plates disappear quickly during post-mortem anoxia and rigor mortis, they must be studied as soon as possible after death and preferably from muscles excised prior to death, and in man from specimens taken for biopsy. However, ante-mortem changes of the motor end-plates accompanying certain morbid processes of the vascular and nervous systems are quite comparable to those which occur during the post-mortem survival period and lead to complete autolysis of the end-plates. This ante-mortem autolysis of end-plates and the localized acute changes in the muscles may be two of the many factors that underlie the malaise, muscle weakness, and paralysis which accompany certain acute infectious processes with toxemia.

12. In evaluating the variable pathologic function and structure of the neuromuscular apparatus in traumatic shock, the factors of intensity and duration of the stimulus, of the time after trauma, and of individual variations of resistance of the living organism must be considered. Fatal traumatic shock is the culmination of a complex sequence of events in the pathologic structure and functioning of the living organism that may be sudden or slow in onset. When traumatic shock is induced in unanesthetized rats in the Noble-Collip drum there are profound pathologic changes in the peripheral nervous system manifested at the neuromuscular apparatus which heretofore have not been studied.

13. It is suggested that the therapeutic agents used in traumatic shock; namely, debridement, blood plasma, and certain supportive measures, are amply sustained by the pathologic findings of the neuromuscular apparatus and localized packing of blood cells in, and plasma loss from, the small vessels of certain muscles. Probably a study of the

agents that would specifically neutralize the hypersecretion from the motor end-plates during the initial stages of shock would render therapy still more effective than at the present time. The application of certain aspects of the therapy of shock to similar conditions produced by infections, namely, poliomyelitis, warrants experimental investigation.

14. Finally, the evidence suggests that any concept of muscle structure which assumes constancy in the number of so-called fixed sarcomeres composed of rigid membranes is a pure morphologic myth handed down by certain histologists for over 100 years. The cross striations are structural expressions that vary in thickness and in number with the variable chemical composition and concentration of the microcapillary muscle fiber. In other words, the variable cross striations are morphologic resultants of the different changes in muscle metabolism.

Sincere gratitude is expressed for help in the laborious task of teasing muscle to J. Schmitz, J. Keyes, E. Socoloff, Miss E. Downer, J. Sweeney, C. Saribalis, and especially to 105 members of the 1944 freshman class who contributed collectively over 5000 man-hours in the teasing survey of muscle connected with this and related problems.

Addendum

Subsequent to the acceptance of this paper for publication, the term *neurosomes* has been used to designate the gold-impregnated inclusion masses discharged from the motor end-plates into and between muscle fibers during thermal shock of rats [68] and chameleons.

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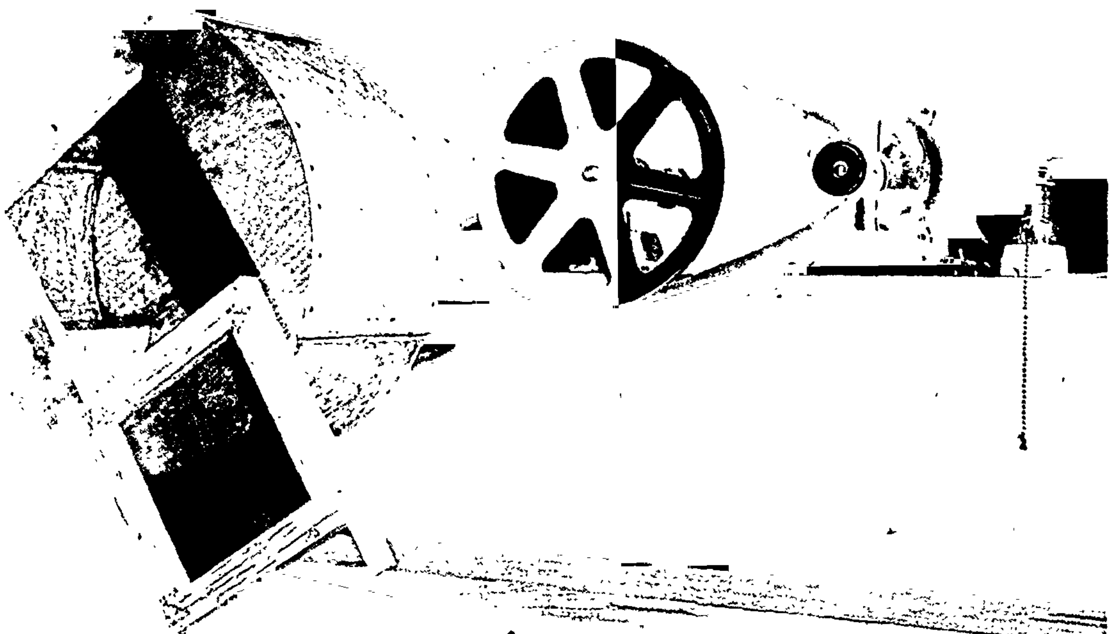
[*Illustrations follow*]

DESCRIPTION OF PLATES

Plate 157

FIGS. 1 and 2. The lateral and upper aspects of the motor-driven Noble-Collip drum. There is a single, circular, galvanized iron drum borne by a short, externally fixed central shaft running on ball bearings. On this shaft are wheels of various size for the driving belt. The drum has an inside diameter of 15 inches and a depth of 8 inches. The two projections on the inside of the drum are blunt, triangular in shape on cross section, with a height of 2 inches and a base of 3 inches. The outside of the drum has a hinged window, part of which is covered by heavy cellophane so that observations may be made.



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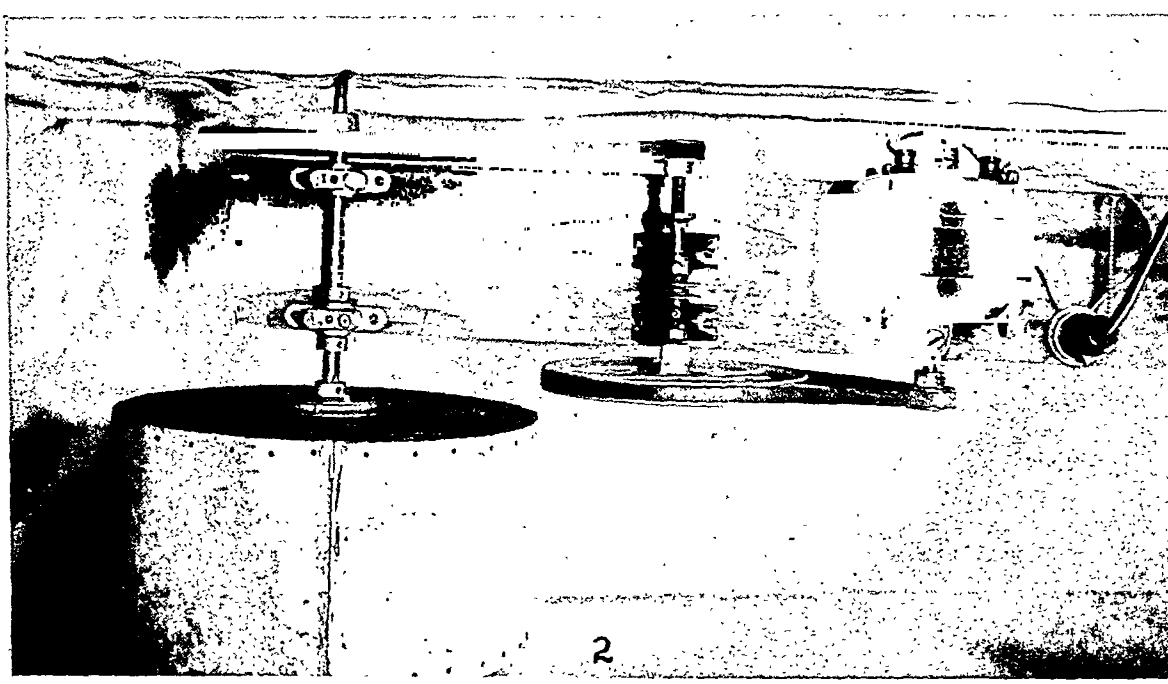

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Carey, Massopust, Zeit,
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Neuromuscular Apparatus and Traumatic Shock

Plate 158

The photomicrographs of Plates 158 to 178 are from teased whole muscle fibers (gastrocnemius muscle) and motor end-plates of the white rat (*Mus norvegicus*), previously prepared by the gold technic. The photographs were direct contact prints from the negatives which were exposed through the microscope and not subjected to subsequent enlargement. They may, therefore, be compared with those of the white rat and the chameleon previously published. In the plates, "epa" designates the epilemmal axon; "hya," the hypolemmal axon; and "Kg.," masses of extra-axonic Kühne's granules. There has been no retouching of either negatives or prints. Traumatic shock was produced in unanesthetized rats in the Noble-Collip drum. Unless otherwise specified, each animal was subjected to 800 revolutions at the rate of 40 r.p.m.

FIG. 3. Sprays of medullated nerve fibers and motor end-plates in the gastrocnemius muscle 5 minutes after the rat had been subjected to trauma. Some of the end-plates are replaced by fine granules and others are completely absent. The epilemmal axons in certain places, therefore, are denuded of their hypolemmal nerve terminals. In some locations elongated streamers of black, gold-staining substance are found. This material accumulates both within and without the muscle fibers during the period that the motor innervation is depleted of its gold-staining substance. This streamer, Kg., has been broken into segments. × 125.

FIG. 4. Sprays of medullated nerve fibers and motor end-plates of variable size from a relatively normal gastrocnemius muscle from a rat of the control series subjected for 15 minutes to nembutal anesthesia. It is necessary for animals used as relatively normal controls to designate either the method of anesthesia or the mechanical method of death such as bleeding or decapitation, because of the high degree of morphologic sensitivity of the motor-end plates to any physical or chemical agent. × 125.

FIG. 5. Sprays of medullated nerve fibers, motor end-plates, and dark masses of axonic material from the gastrocnemius muscle 5 minutes after it had been subjected to trauma. Some of the end-plates are absent, others are granular and in direct relationship with certain dark-staining masses of extruded axonic material. × 125.

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PLATE 159

FIGS. 6 and 7. Sprays of medullated nerve fibers and motor end-plates showing variable degrees of projection of axonic material into the muscle 5 minutes subsequent to trauma. In some places there is a fine filament between the epilemmal and hypolemmal axon. In others there is a small droplet at this location. Some of the end-plates are retracted into dark-staining masses and others are expanded. Elongated streamers of projected axonic material (Kg.), which represent one stage in the loss of motor innervation, are clearly evident (Fig. 7) in direct continuity with the altered motor end-plates. × 125.

FIG. 8. Sprays of medullated nerve fibers of the gastrocnemius muscle completely denuded of their hypolemmal axons 1 hour after the muscle had been subjected to trauma. The liquefied motor end-plates are identified in some locations by the fine granular remains of the plate, while in others no evidence of the dissolved end-plate is observed. In places the related cross striations have likewise undergone a granular alteration and liquefaction. In certain locations the epilemmal axon terminates in a sharp point and in others in a rounded knob. The change in the motor innervation of the traumatized muscle begins distad at the motor end-plate and progresses in a centripetal direction in the epilemmal axons. The granular degeneration, beading, and fragmentation of the epilemmal axon therefore extend in a central direction and away from the altered end-plates after muscle trauma. × 125.

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PLATE 160

FIGS. 9 to 12. Sprays of medullated nerve fibers and motor end-plates of variable sizes in the gastrocnemius muscle 15 minutes after the animal had been subjected to trauma. Dark-staining oval droplets and elongated streamers of axonic material are in direct relation to the traumatized end-plates in many locations. Some end-plates are retracted, others are expanded, and still others are completely liquefied. The normal granular sole of Kühne is replaced in some locations by an abnormal accumulation of the axonic material both within and without the motor end-plate. In some locations the muscle fibers have the histologic characteristics of Zenker's waxy degeneration. × 125.

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PLATE 161

FIGS. 13 and 14. Sprays of medullated nerve fibers and motor end-plates in the gastrocnemius muscle 15 minutes after the animal was traumatized in the rotary drum. The axonic droplets are found both within and without the motor end-plates. In some plates this axonic material is found in direct continuity with the motor end-plate, and in others separated from the end-plate and projected into the muscle fiber (Fig. 13). These axonic streamers vary from 10 to 150 μ in length and from 2 to 50 μ in diameter. The axonic droplets are unipolar, bipolar, and irregular in arrangement in relation to the highly permeable motor end-plate. Waxy degeneration of the muscle fibers is evident in some locations. $\times$ 125.

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PLATE 162

FIGS. 15, 16, and 17. Sprays of medullated nerve fibers and motor end-plates in the gastrocnemius muscle 15 minutes subsequent to traumatization in the rotary drum. There are variable degrees of loss of motor innervation by the abnormal pouring out of axonic material both within and without the muscle fibers. The end-plates are either expanded or retracted, and in some locations completely absent. Progressive steps in the transmission of the dark-staining axonic material may be traced through the epilemmal axon, hypolemmal axon, and external to the end-plate into the muscle fiber. Projected axonic material is either unipolar or bipolar in arrangement, or irregular in its disposition in relation to the motor end-plate. Some of the traumatized muscle fibers (Fig. 15) form fusiform structures and exhibit an alteration of the myoplasm similar to that found in waxy degeneration produced by lactic acid. × 125.

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Plate 163

Figs. 18, 19, and 20. Sprays of medullated nerve fibers and motor end-plates in the gastrocnemius muscle 15 minutes subsequent to traumatization in the rotary drum. There is an irregular dilatation and constriction of the epilemmal axons (epa). The hypolemmal axons (hya) are either retracted or expanded. Some of the end-plates (Fig. 19) have an accumulation of axonic material in the center of the plate which forms a hub-like appearance. In the expanded plates there is a diminution of the granular sole of Kühne which is replaced by a unipolar droplet of this granular material (Figs. 18 and 19, Kg.). In other locations the end-plate has a large, oval-shaped droplet of the projected axonic material (Fig. 20, Kg.). × 350.

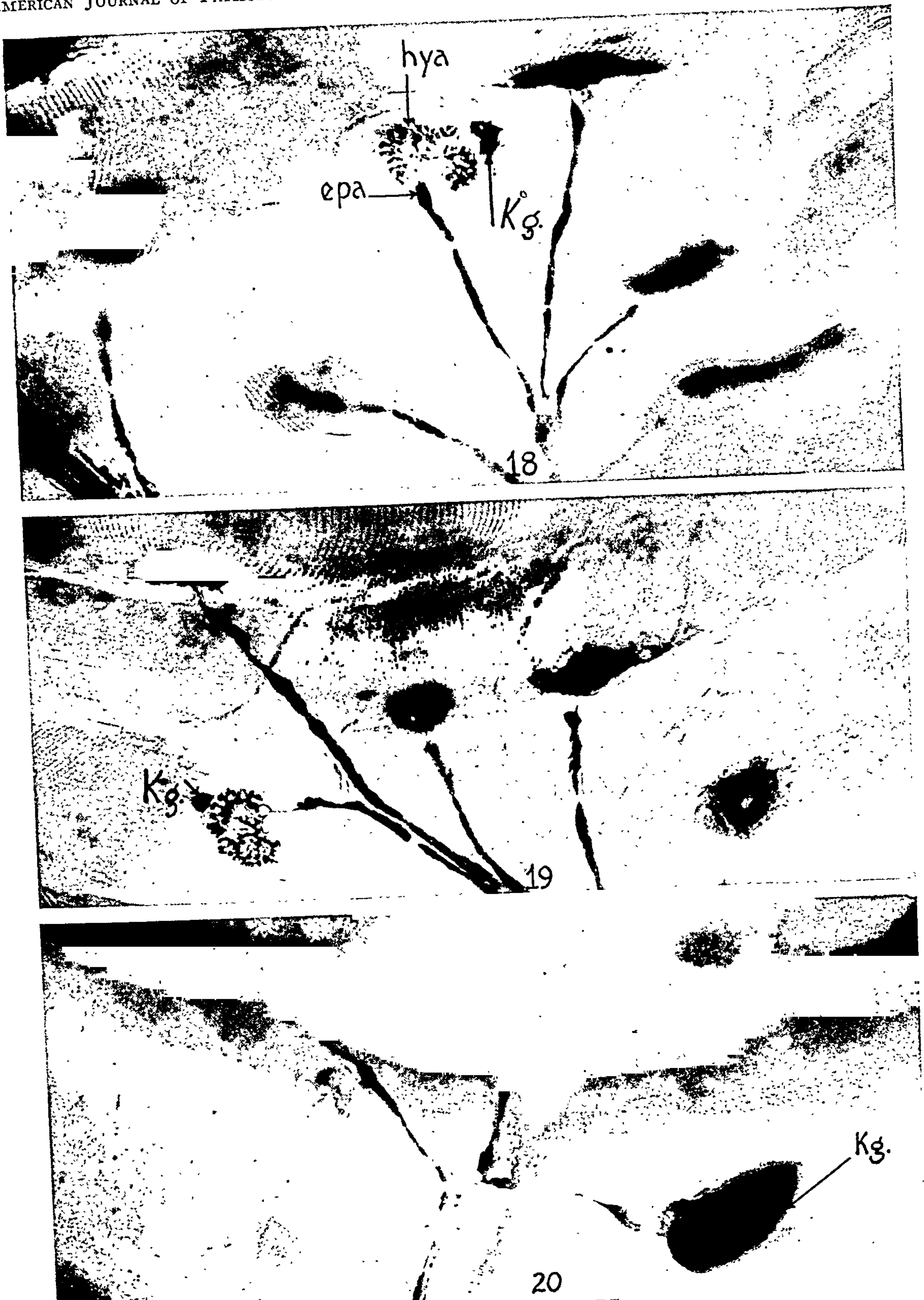

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PLATE 164

FIGS. 21, 22, and 23. Sprays of medullated nerve fibers and motor end-plates in the gastrocnemius muscle 15 minutes after subjection to trauma. There is an irregular dilatation and constriction of the epilemmal axons (epa). The hypolemmal axons (hya) are either retracted or expanded. The irregular accumulation and arrangement of the projected axonic material (Kg.) are clearly evident. These axonic droplets secreted by the motor end-plates take an intensive impregnation with gold and have either a unipolar (Fig. 22) or a typical bipolar arrangement (Fig. 23), or are irregular in arrangement and form elongated streamers (Fig. 21). These elongated streamers may or may not be cross-striated. In some places the cross striations of the axonic streamers conform with the periodicity of those of the muscle fibers. In other locations there is no agreement in the periodicities of these variable striations. × 350.

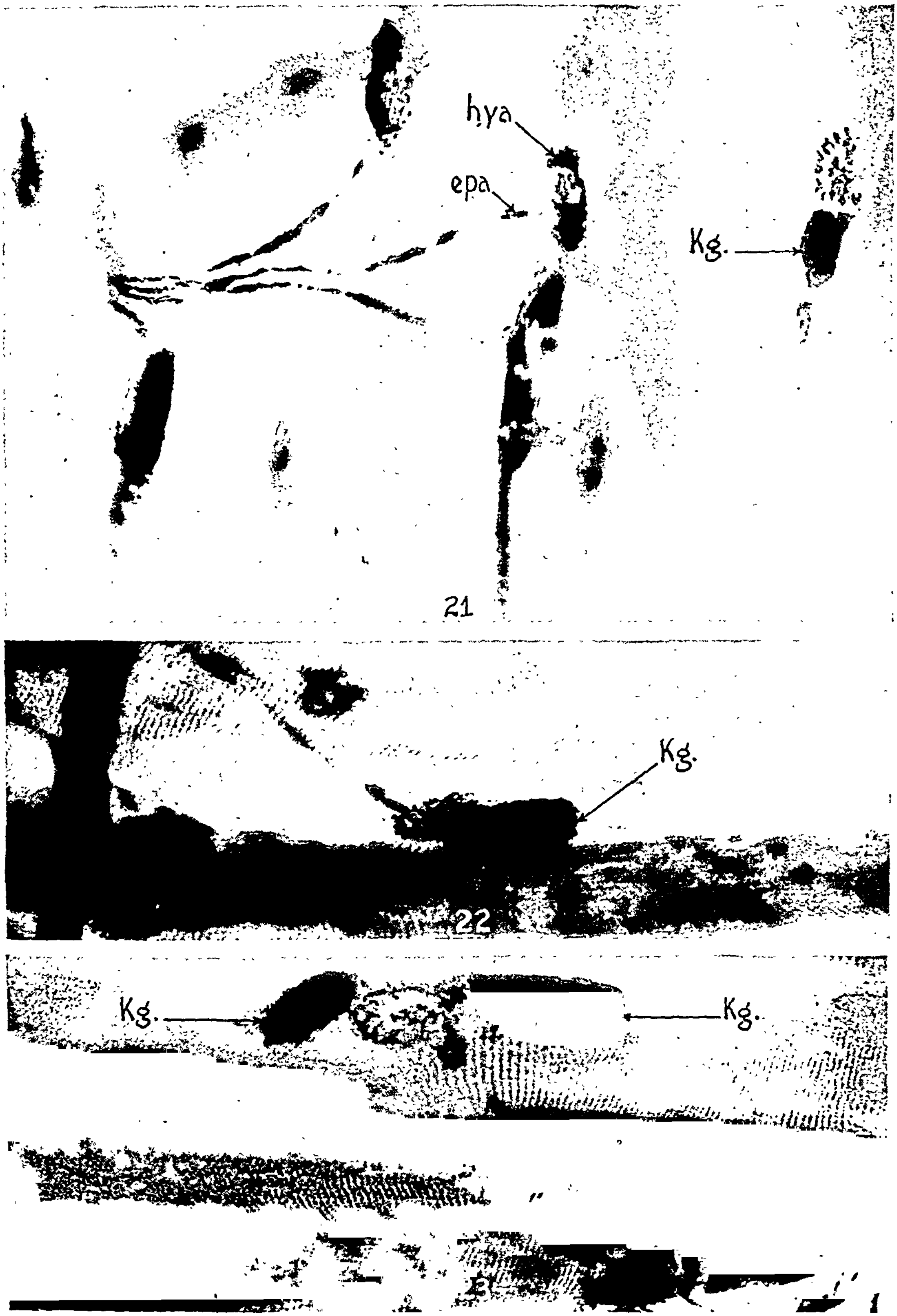

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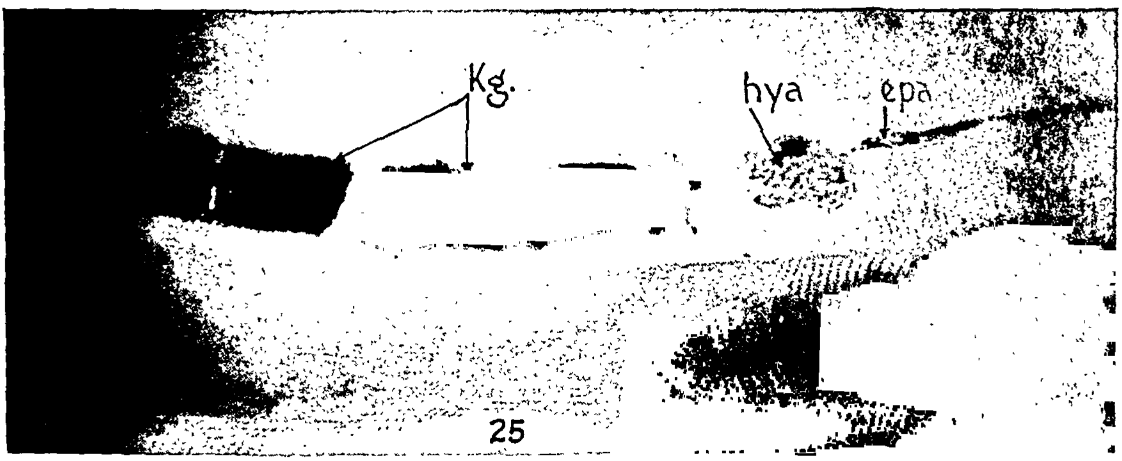
PLATE 165

FIGS. 24, 25, and 26. Sprays of medullated nerve fibers and motor end-plates in the gastrocnemius muscle 15 minutes subsequent to traumatization. There is a semicircular arrangement of the dark mass of Kühne's granules (Fig. 24, Kg.) about the motor end-plate. An irregular arrangement of Kühne's granules is present at the pole opposite the one in which there is a massive accumulation of these granules. To the left of the motor end-plate there is a unipolar arrangement of the large masses of Kühne's granules (Fig. 25, Kg.). These masses that take an intense impregnation with gold represent an accumulation of axonic material that drains out of the motor end-plate. There is a bipolar arrangement of the large masses of Kühne's granules to the left and right of the motor end-plate (Fig. 26, Kg.), and a large dark droplet of axonic material in the center of this motor end-plate. × 350.



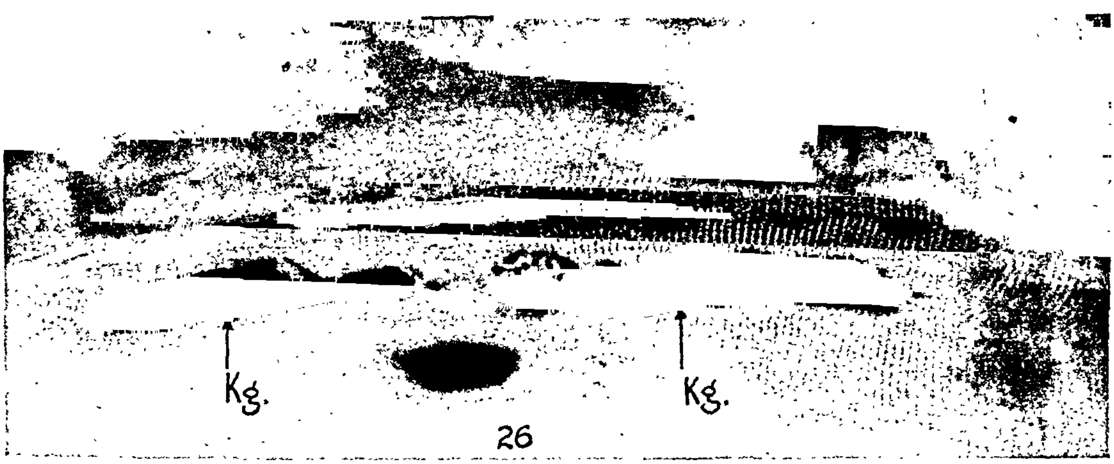


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Plate 166

Figs. 27, 28, and 29. Sprays of medullated nerve fibers and motor end-plates in the gastrocnemius muscle 15 minutes subsequent to traumatization. Different stages are found in the transmission of axonic material through the beaded epilemmal axons (epa), hypolemmal axons (hya), and dark-staining masses of Kühne's granules (Kg.) found at variable distances from the motor end-plates in the muscle fibers. The dark-staining axonic material is found in the center of the end-plate, in a unipolar position external to the end-plate, as well as in a bipolar arrangement (Fig. 27). Small fusiform masses of axonic material (Kg.) are found projected from the lower motor end-plate (Fig. 27), and extend into the muscle fiber a considerable distance away from the end-plate. Some of these dark-staining fusiform masses are cross-striated. Others have a serrated appearance around the periphery. Concomitant with the projection of these axonic masses in the muscle fiber, there is in some locations a depletion of the epilemmal and hypolemmal axons. There is a central appearance of axonic material in the motor end-plate and a unipolar arrangement of this material (Fig. 28, Kg.). There is a large ovoid droplet of axonic material related to the motor end-plate below and to the right of the motor tree (Fig. 29, Kg.). These dark-staining masses of Kühne's granules (Kg.) appear to represent an abnormal secretion of nervous liquid into the muscle fiber. The abnormal appearance of this nervous material is evanescent and the material is subsequently dissolved; therefore large amounts of muscle material must be teased in order to detect the abnormal appearance of this nerve material in muscle. × 350.

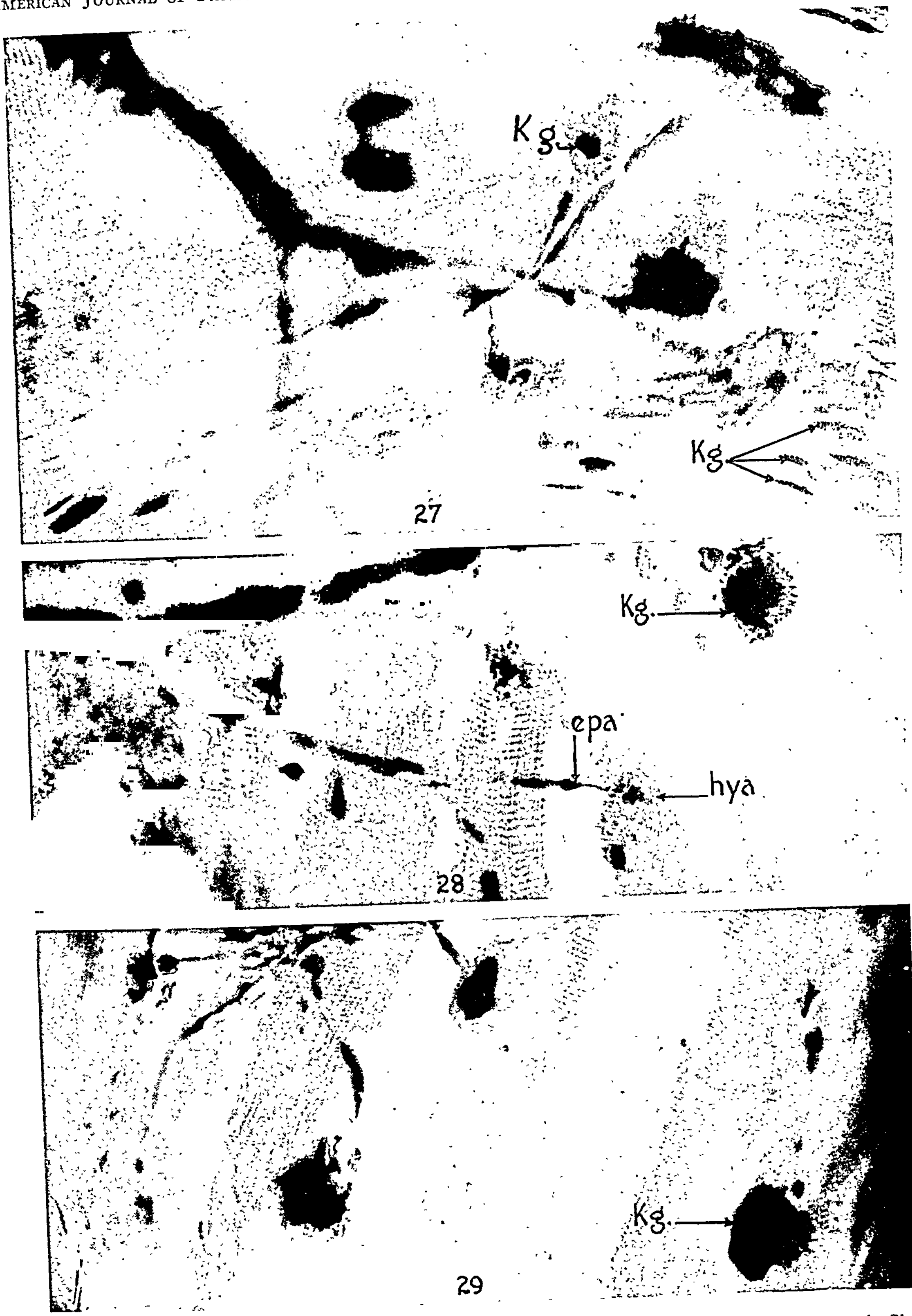

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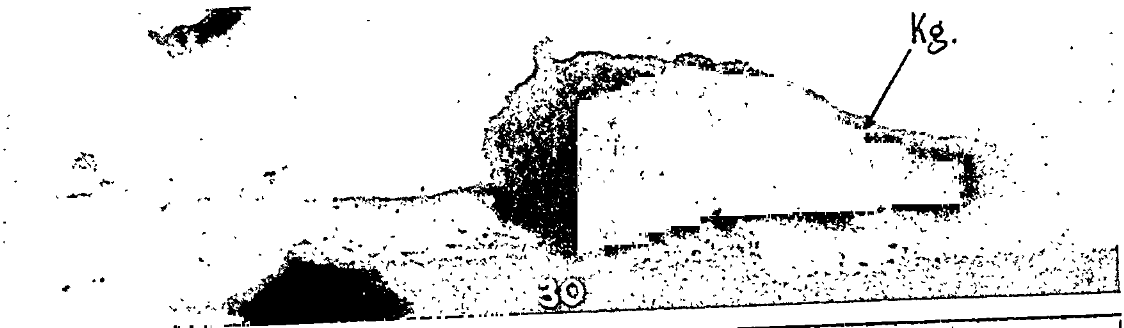
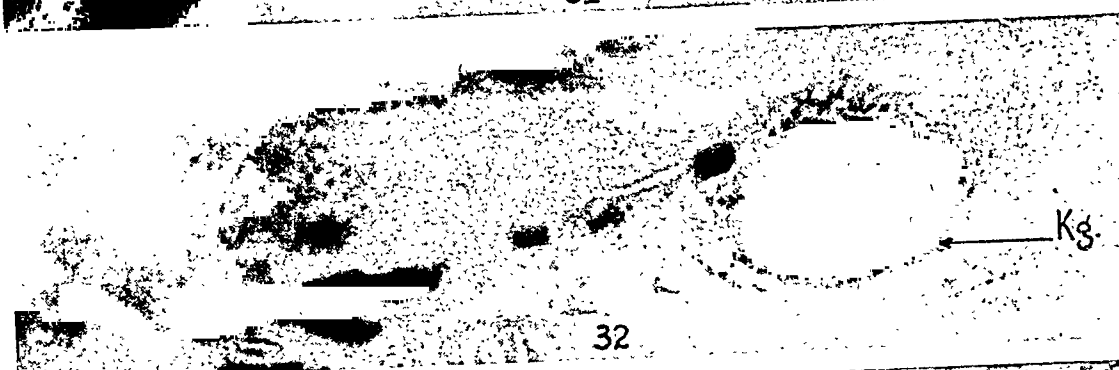
PLATE 167

FIGS. 30 to 33. Sprays of medullated nerve fibers and motor end-plates in the gastrocnemius muscle 15 minutes subsequent to traumatization. Different stages are found in the transmission of axonic material through the beaded epilemmal axons (epa), hypolemmal axons (hya). and dark-staining masses of Kühne's granules (Kg.). There is an oblong mass of Kühne's granules which overlies the motor end-plate (Fig. 30. Kg.). A dark ovoid mass of Kühne's granules is found in a unipolar position to the motor end-plate opposite to the pole where the union of the epilemmal axon is found (Fig. 31, Kg.). There is a flattened spherical mass of Kühne's granules at one pole of the motor end-plates (Figs. 32 and 33). × 750.





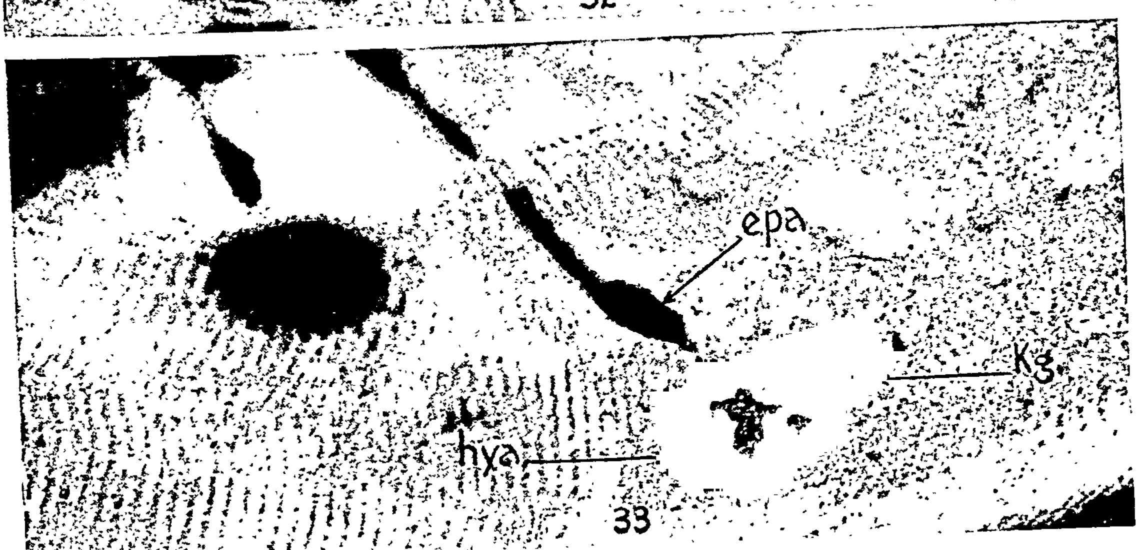


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Plate 168

Figs. 34 to 38. Sprays of medullated nerve fibers and motor end-plates in the gastrocnemius muscle 30 minutes subsequent to traumatization. Different stages are found in the transmission of axonic material through the beaded epilemmal axons (epa), hypolemmal axons (hya), and dark-staining masses of Kühne's granules (Kg.). There is a large, dark droplet of Kühne's granules in the center of the motor end-plate (Fig. 34, Kg.), and a small spherical droplet of Kühne's granules external to the motor end-plate and at the pole opposite to that of the continuity with the epilemmal axons (Fig. 35, Kg.). A bipolar arrangement of large oblong masses of Kühne's granules is extrinsic to the hypolemmal axons of the end-plate (Fig. 36, Kg.). There is a unipolar arrangement of the large, dark, discoidal mass of Kühne's granules at the pole opposite to that which is continuous with the epilemmal axon (Fig. 37, Kg.). An irregular arrangement of the large, dark masses of Kühne's granules is shown about the motor end-plate (Fig. 38, Kg.). × 750.

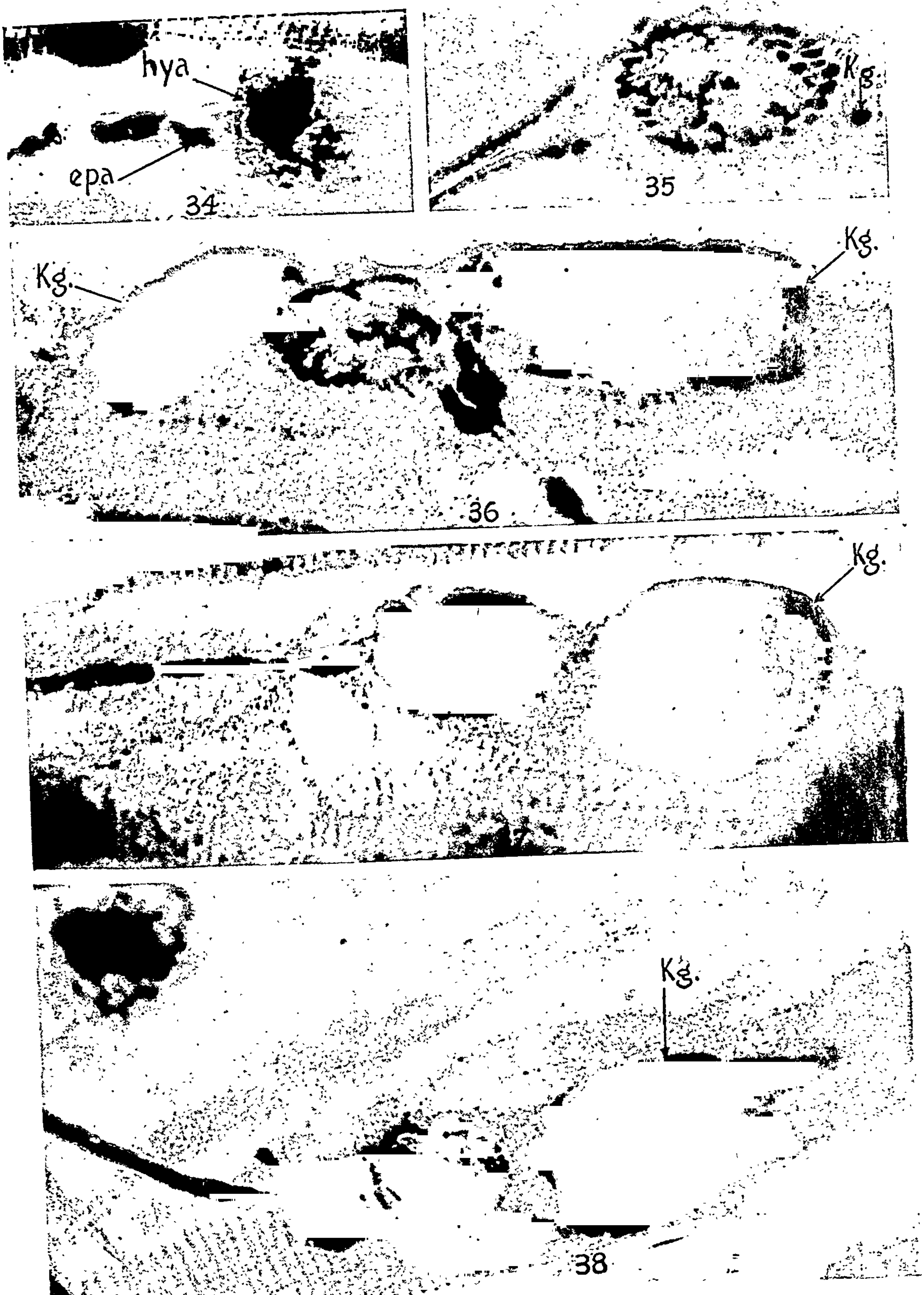

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PLATE 169

FIGS. 39, 40, and 41. Motor end-plates from gastrocnemius muscle of an unanesthetized rat 15 minutes after subjection to trauma. An elongated streamer of Kühne's granules (Kg.) is observed to the right (Fig. 39) of the motor end-plate. Discrete and fusiform masses of these granules are projected to the right (Figs. 40 and 41) of each motor end-plate. In some places these masses of granules are homogeneous, and in others they have a cross-striated structure. $\times$ 750.

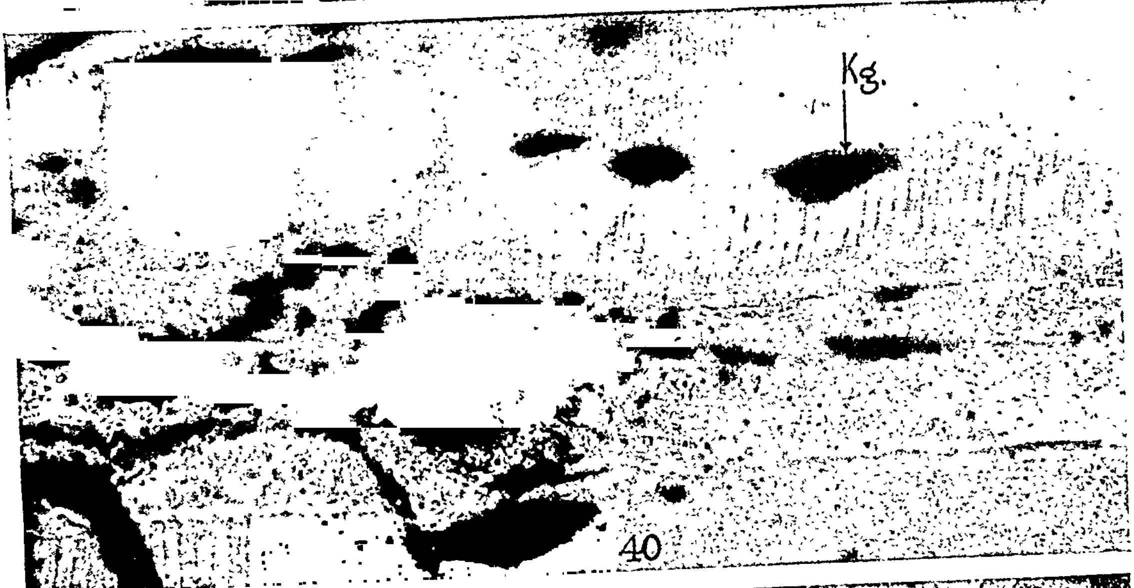

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Plate 170

Figs. 42 to 45. Motor end-plates from the gastrocnemius muscle of an unanesthetized rat 15 minutes after subjection to trauma. An elongated streamer of Kühne's granules (Fig. 43, Kg.) is observed extending downward from the dark, retracted end-plate. Some end-plates are in a state of abnormal retraction (Fig. 42) and take an intense impregnation with gold. Other end-plates are relatively normal and have a clear, halo-like space between the hypolemmal axons and the rim of Kühne's granules (Fig. 44). Abnormal retraction and hyperchromatophilia for gold are the first effects of the local injection of lactic acid experimentally into muscle. This first phase of retraction and hyperchromatophilia is quickly followed by the second phase of expansion, projection of axonic material into the muscle, disintegration, and complete liquefaction. × 750.

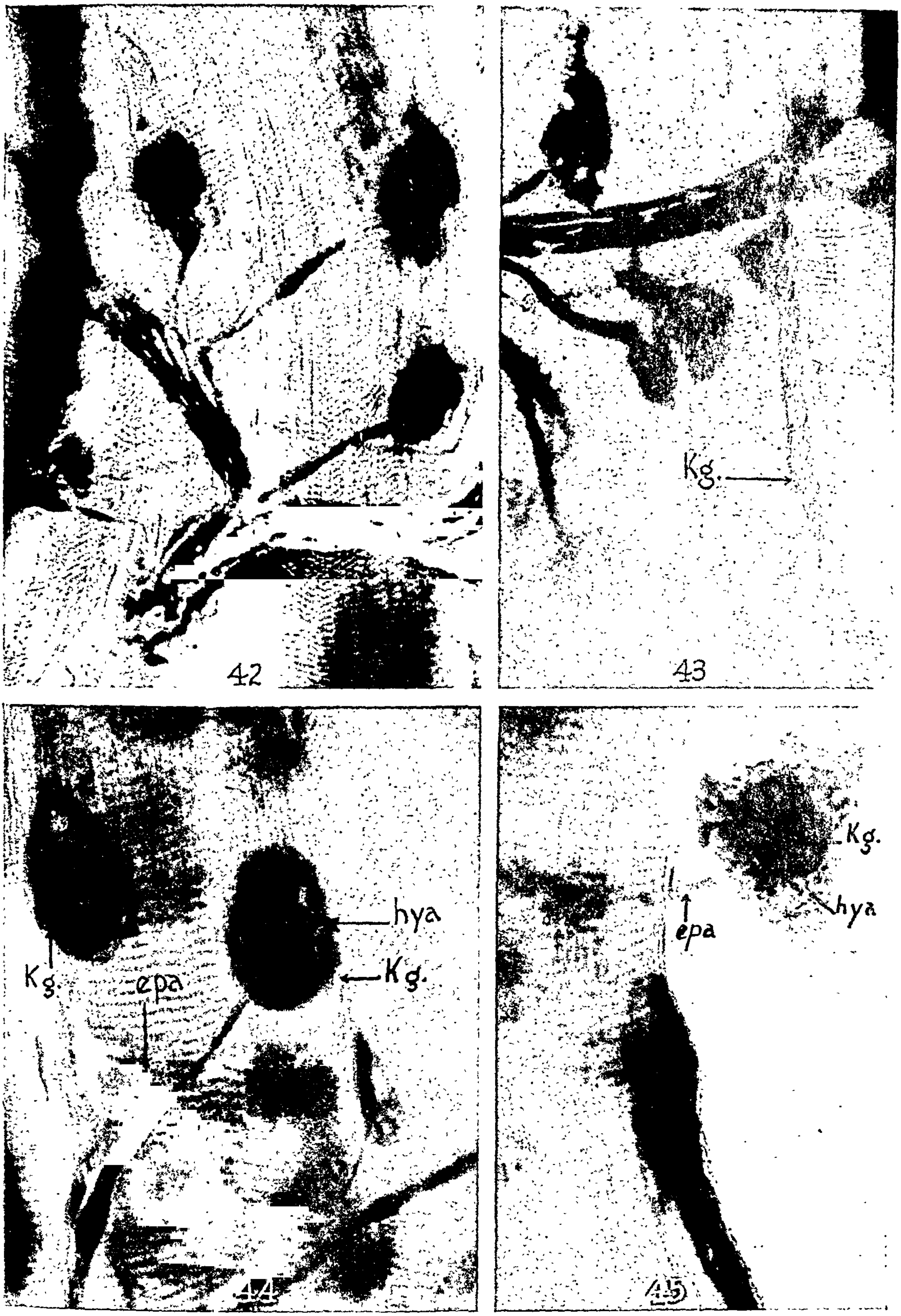

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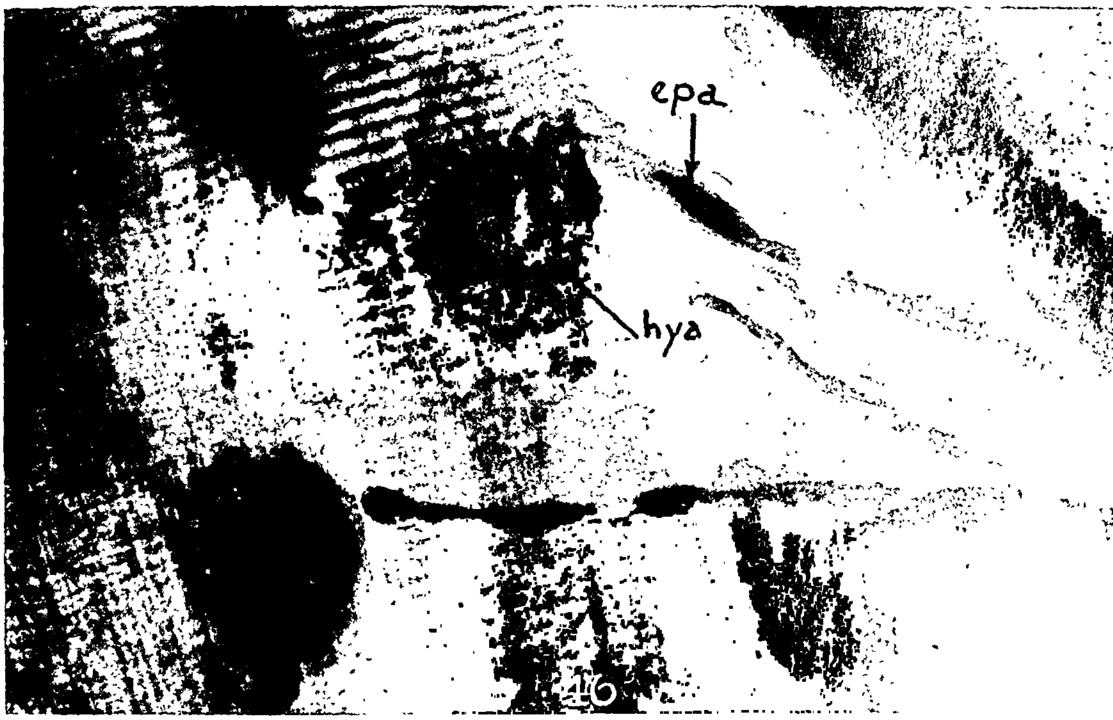
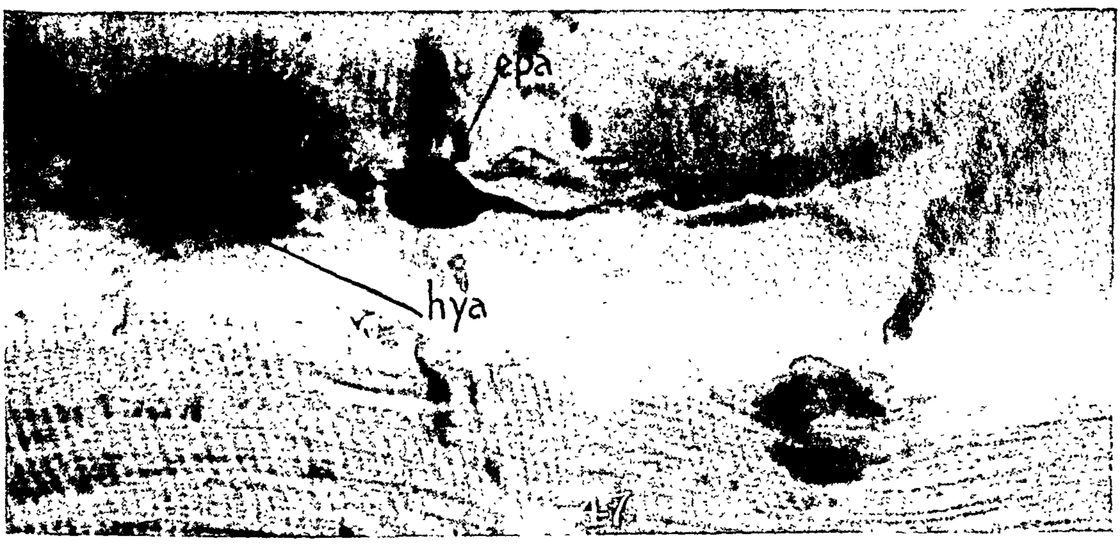
PLATE 171

FIGS. 46, 47. and 48. Motor end-plates from the gastrocnemius muscle of an unanesthetized rat 15 minutes after subjection to trauma. There is an irregular moniliform pattern of the epilemmal axons (epa). Some of the hypolemmal axons (hya) of the motor end-plates are relatively normal and have variable degrees of expansion and quantitative condensation of Kühne's granules (Fig. 46). In some places there is a diminished affinity of the end-plate for gold with a corresponding granular degeneration of both the motor end-plates and muscle fibers (Fig. 48). One end-plate to the left has a central island of Kühne's granules (Fig. 48). × 750.

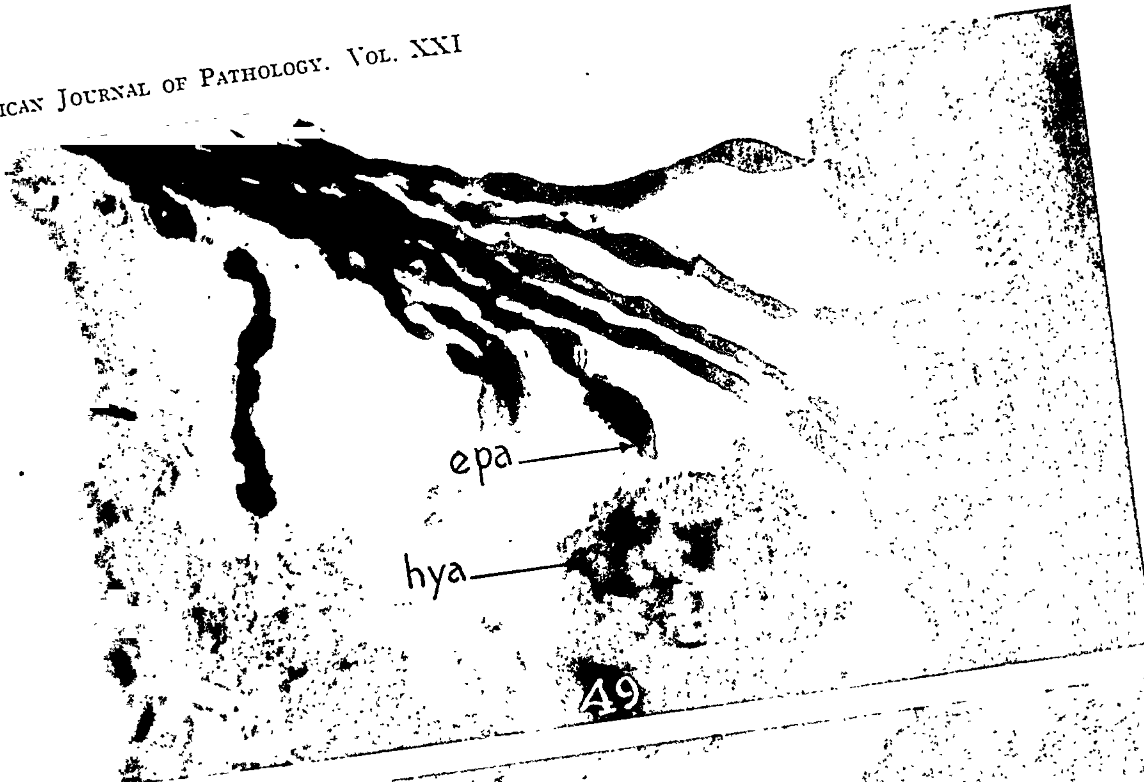

epa
hya
46

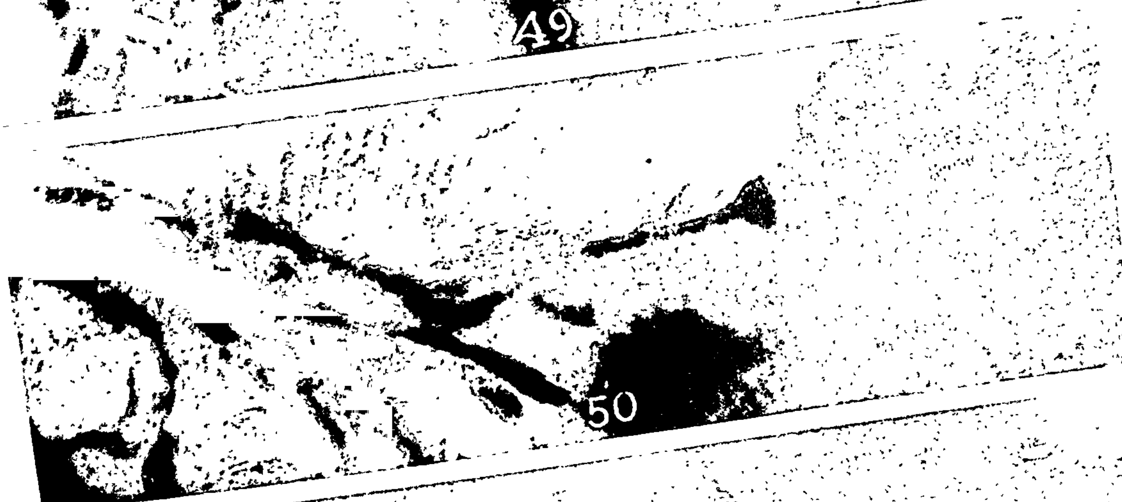

epa
hya
47


48

PLATE 172

FIGS. 49, 50, and 51. Motor end-plates from gastrocnemius muscle of an unanesthetized rat 15 minutes after traumatization. There is an irregular moniliform pattern of the epilemmal axons (epa). The hypolemmal axons (hya) of the motor end-plates have variable degrees of expansion and liquefaction. In some of the motor end-plates there is practically complete disappearance of the hypolemmal axons and of the granules of Kühne. These morphologic changes in the motor end-plates produced by traumatic shock are comparable to those caused by the injection experimentally of lactic acid directly into living, nerve-intact muscle. × 750.


epa
hya
49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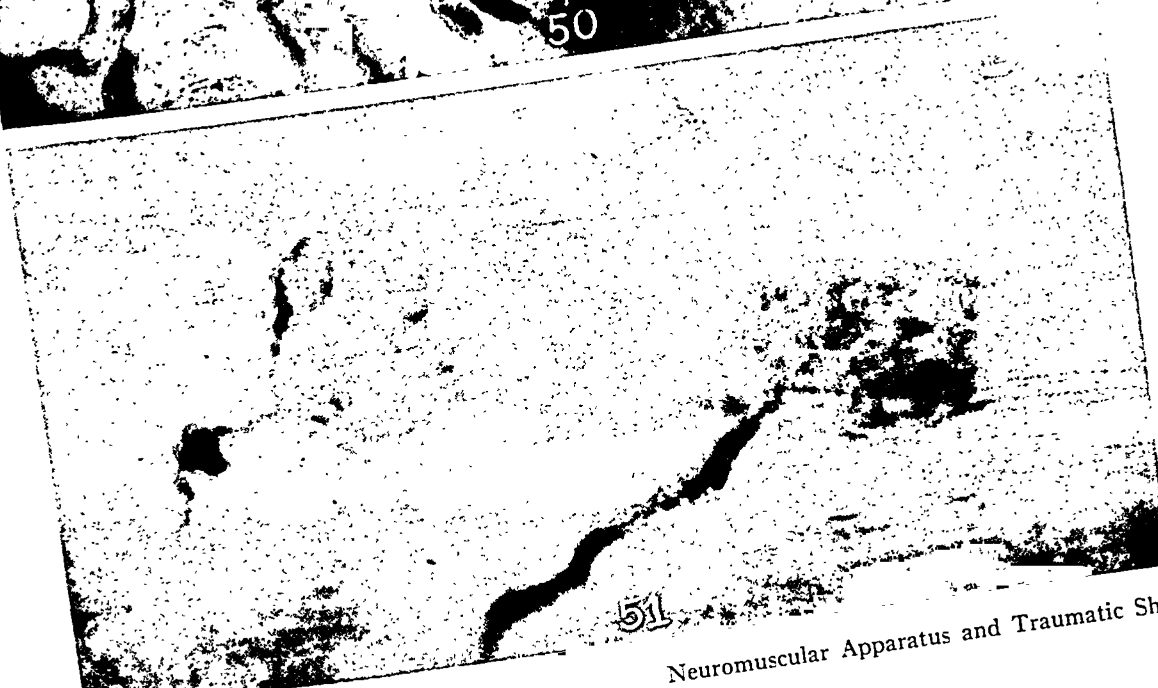
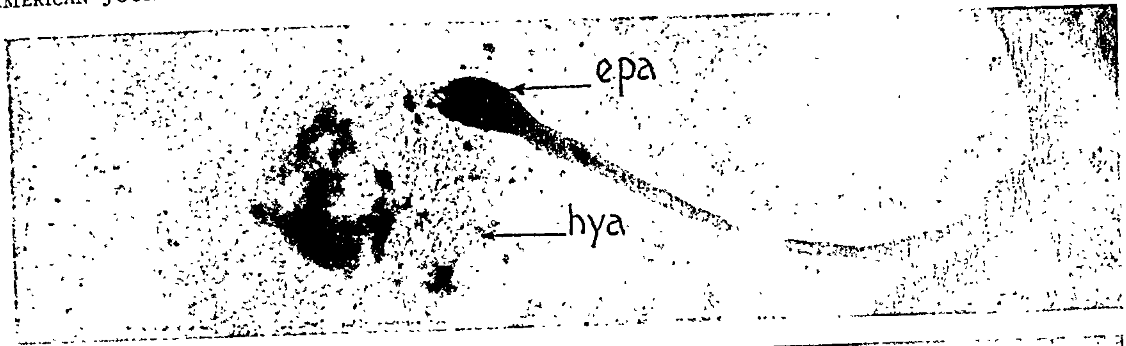

51

PLATE 173

FIGS. 52, 53, and 54. Motor end-plates from the gastrocnemius muscle of ar unanesthetized rat 15 minutes after subjection to trauma. There is an irregular moniliform pattern of the epilemmal axons. An especially large bulb of the epilemmal axon (epa) is found at its junction with the hypolemmal axons (hya) (Fig. 52). These hypolemmal axons are undergoing liquefaction (Fig 52). There is an irregular dispersal of granules in relation to some of the disintegrating motor end-plates (Fig. 53). In other locations of the same muscle the hypolemmal axons have completely disappeared. There is, likewise, a complete disappearance of the granules of Kühne (Fig. 54). The clear, rounded and oval areas in the location previously occupied by the hypolemmal axons are the outlines of the nuclei related to the motor end-plate in the muscle fiber (Fig. 54, Nu). × 750.



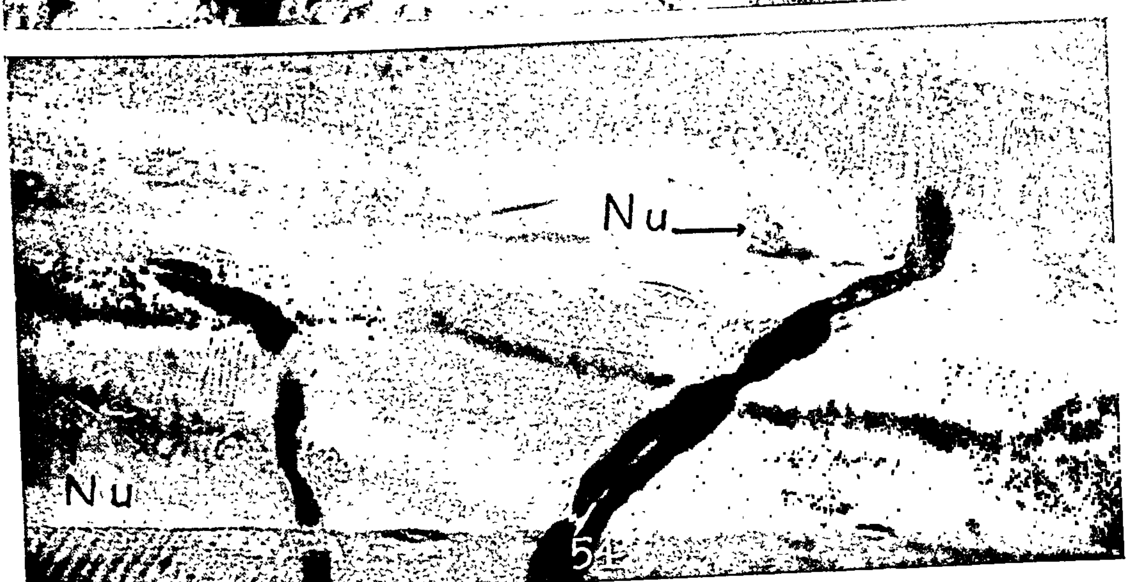


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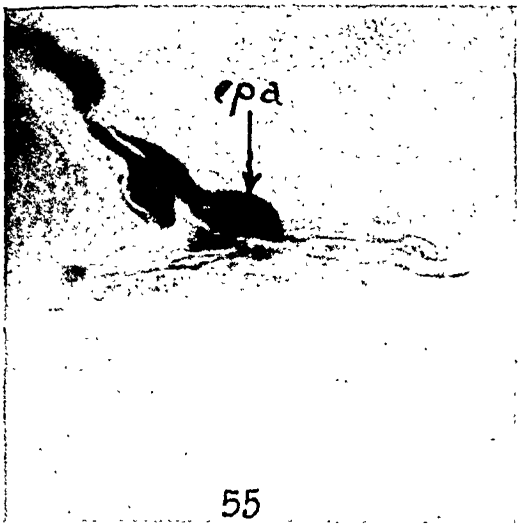
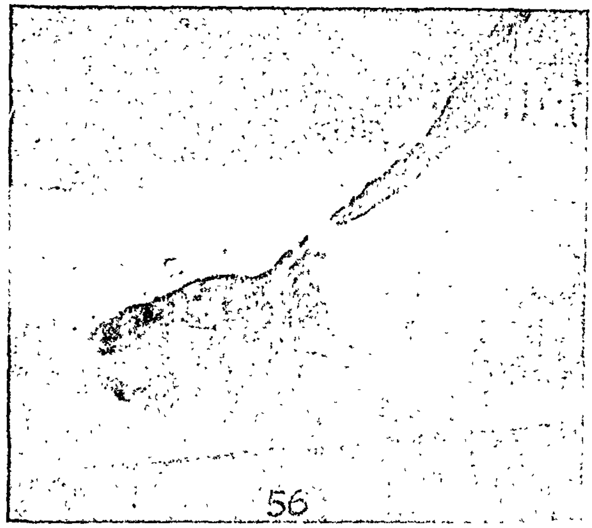
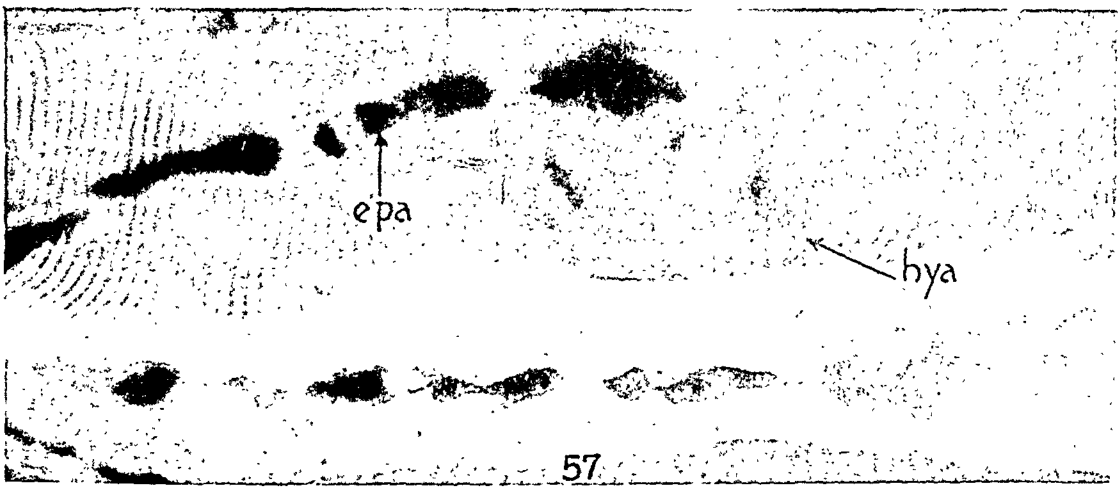
PLATE 174

FIGS. 55 to 58. Motor end-plates from the gastrocnemius muscle of an unanesthetized rat 15 minutes after subjection to trauma. There is an irregular moniliform pattern of the epilemmal axons (epa). There is a complete disappearance of the hypolemmal axons of the motor end-plates in some locations (Figs. 55, 56, and 58). Remnants of the hypolemmal axons (hya) and granules of Kühne are found in other locations (Fig. 57). In certain areas the epilemmal axons are undergoing a complete granular degeneration (Fig. 58). × 750.







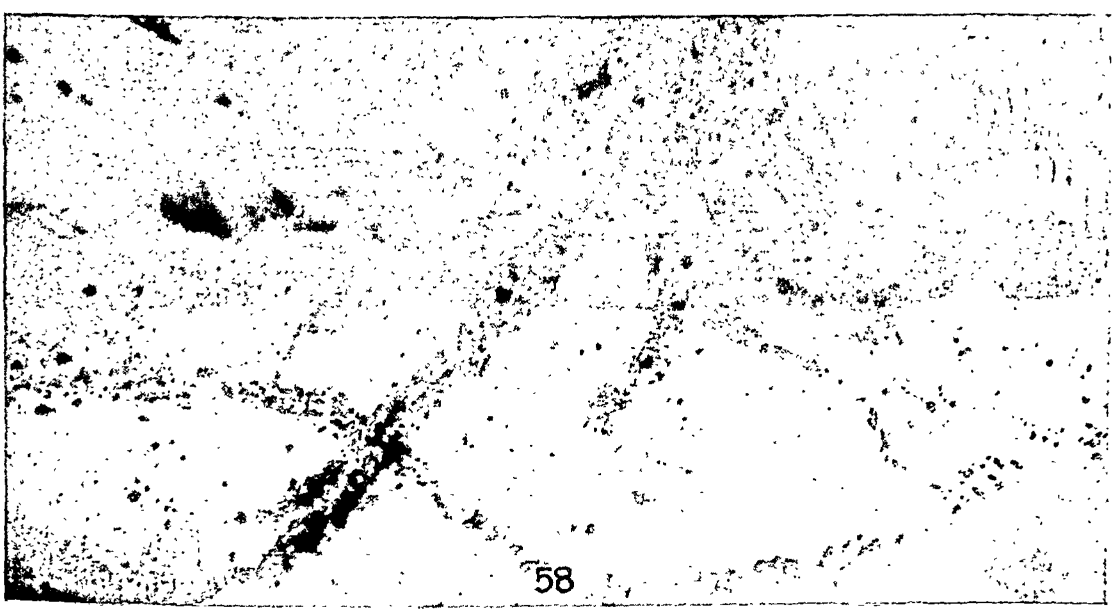


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PLATE 175

FIGS. 59, 60, and 61. Gastrocnemius muscle from an unanesthetized rat 15 minutes after subjection to trauma. Sprays of medullated nerve fibers (Fig. 59) are denuded of motor end-plates. Some of the muscle nuclei exhibit increased visibility and there are irregular coagulation bands of muscle tissue, some of which are opaque and others finely cross-striated. In some locations the blood vessels are dilated and packed with red blood cells (Fig. 60), with incipient perivascular infiltration of polymorphonuclear leukocytes. The morphologic lesion of the neuromuscular apparatus produced by trauma is a structural expression of the profound alteration in the underlying biochemical processes of nerve and muscle. In still other locations the cross striations of the muscle fibers are replaced by a dense granulation that forms oblong or fusiform structures (Fig. 61). These acute degenerative changes are comparable to those produced by the local injection of lactic acid into the muscle. × 125.

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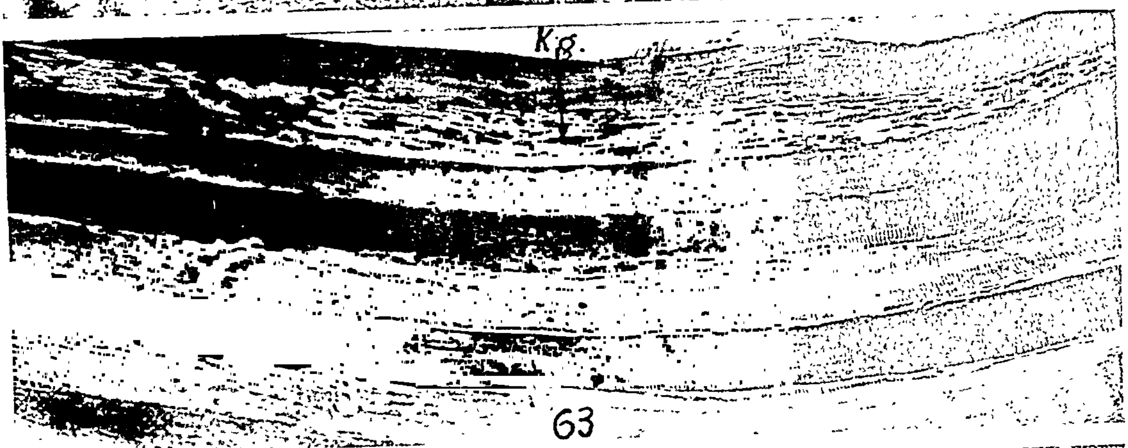
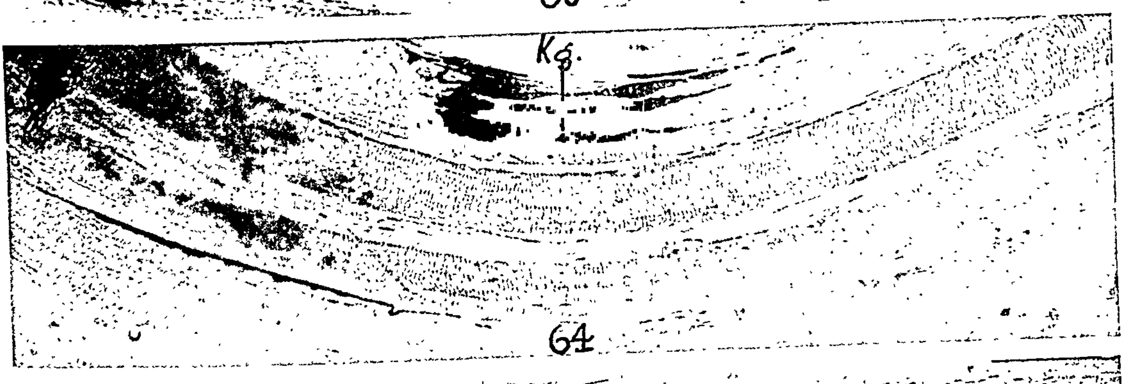
Neuromuscular Apparatus and Traumatic Shock

PLATE 176

FIGS. 62 to 65. Gastrocnemius muscle from an unanesthetized rat 15 minutes after subjection to trauma. Sprays of medullated nerve fibers are found, some of which are denuded of their motor end-plates. In one of the fibers (Fig. 62) there are oblong and fusiform gold-impregnated masses scattered throughout the myoplasm. This material appears in the muscle fiber coincident with the disappearance of the end-plate and the depletion of axonic materials from the epilemmal axons. Some of the muscle fibers retain their cross-striated structure and others show an intermingling of the cross striations and the gold-impregnated axonic substance (Figs. 62 to 65, Kg.). This axonic substance may or may not be cross-striated. If striated, it may or may not have the periodicity of its bandings coincide with that of the cross striations of the muscle. In certain locations this axonic material may form greatly elongated and fragmented streamers between the muscle fibers. These may be 500 to 1000 μ in length (Figs. 64 and 65, Kg.). This axonic material (Kg.) may be found either within or without the sarcolemma of the muscle fiber. $\times$ 125.





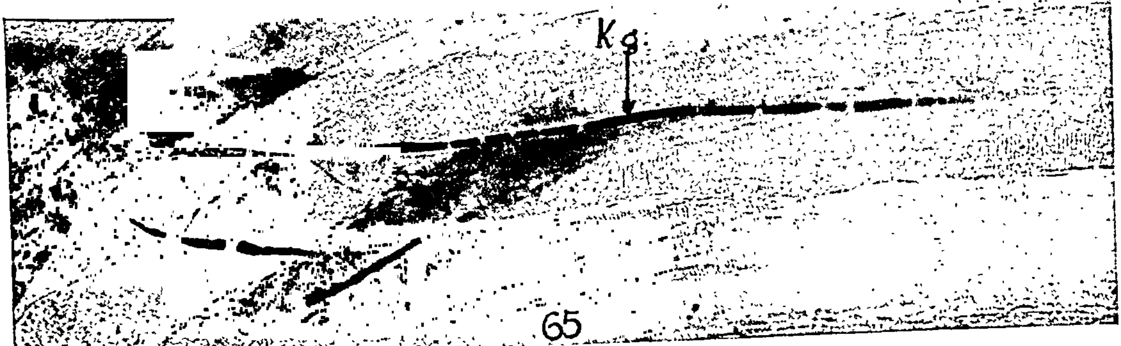


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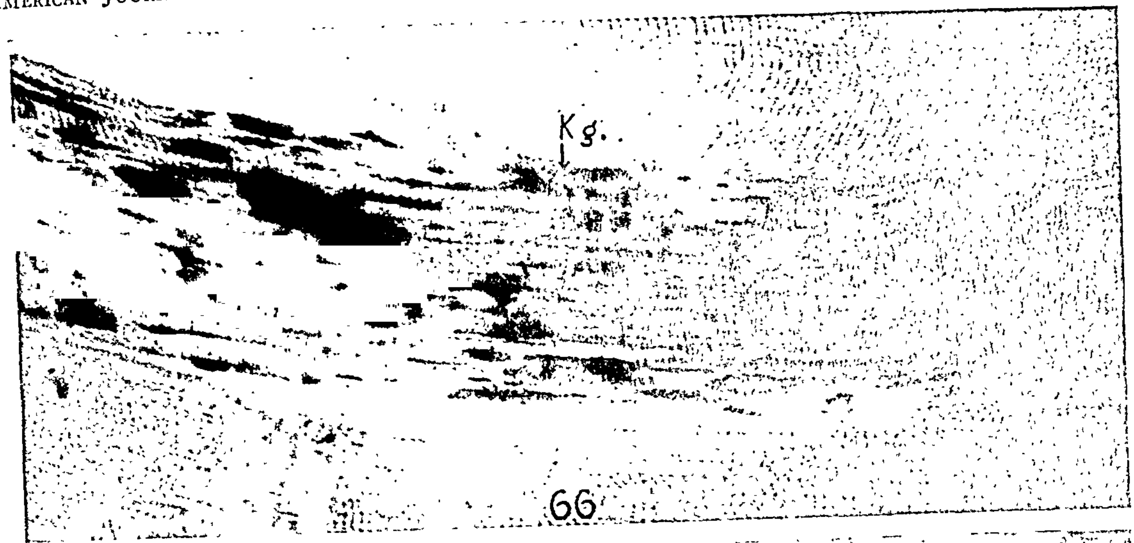
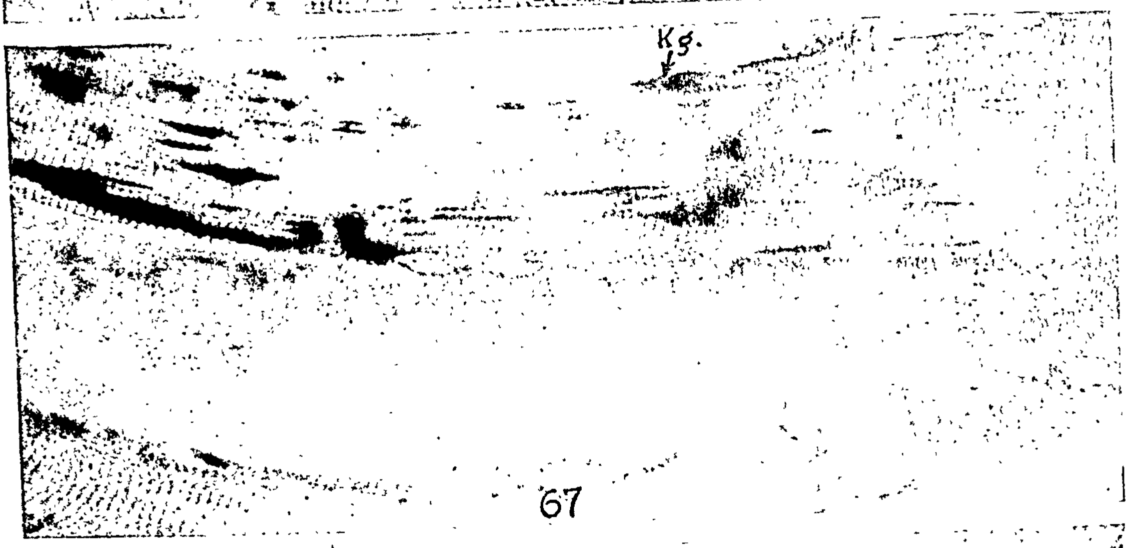
PLATE 177

FIGS. 66, 67, and 68. Gastrocnemius muscle fibers from an unanesthetized rat 15 minutes after subjection to trauma. In some muscle fibers the discharged axonic material (Kg.) that is hyperchromatic for gold is densely crowded into oblong and fusiform masses. In many fibers of the same muscle these gold-impregnated masses are absent. This projection of auriphilous material was not found in the bilateral control muscles from ten rats. These masses are either opaque with serrated borders or cross-striated. Their cross striations may or may not conform with those of the muscle fiber in which they are found. These ephemeral masses of projected axonic material are found only during the time of, and shortly after, muscle trauma. Within 1 to 2 hours after traumatization they disappear. Their appearance in muscle is coincident with that of morphologic depletion of the motor innervation of its axonic material; and their disappearance, with the beginning of granular degeneration of some of the muscle fibers. The local injection of lactic acid into muscle produces a similar structural depletion of motor innervation and the ephemeral appearance of axonic masses in the muscle. The increased acidity, experimentally produced locally in muscle, augments the permeability of the end-plates and structurally exhausts the axonic conduction medium of the innervation of muscle. × 750.





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Neuromuscular Apparatus and Traumatic Shock

PLATE 178

FIGS. 69 to 72. Proprioceptive muscle spindles (Figs. 69 to 71, M.S.) in the gastrocnemius muscle from an unanesthetized rat 15 minutes after subjection to trauma. In some places the sensory and motor terminals of the spindle are fragmented. The afferent sensory axons of the spindle are enlarged and in some places (Fig. 69, D) greatly dilated and hyperchromatic for gold. The epilemmal axons (epa) are denuded of their hypolemmal axons that form the motor end-plates. The increased visibility of the dark nuclei (Fig. 69) of the muscle fibers is comparable to that produced by the experimental injection of lactic acid locally into the muscle. The altered structure of motor and sensory nerve endings and of the muscle fibers and blood capillaries produced by trauma appears to parallel the alteration of metabolism resulting from anoxia produced by the experimental injection of lactic acid locally into muscle. The increased concentration of lactic acid increases the permeability of the end-plate which results in an augmented outflow of the axonic substance into the muscle. The tendon spindle (Fig. 72, T.S.) is composed of branched terminals that end in club-shaped enlargements. There is some evidence of axonic fragmentation. × 125.

Carey, Massopust, Zeit,
Haushalter, Hamel, and Jeub

Neuromuscular Apparatus and Traumatic Shock

CELLULAR REACTIONS TO MYCOLIC ACIDS *

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Mycobacteria may be differentiated from other microorganisms in their content and variety of fatty acids, phosphatides and wax-like substances. The latter, when isolated chemically,[1] are acid-fast and are assumed to be responsible for the staining characteristics of the mycobacteria.[2] These waxy substances, particularly those of the tubercle bacillus, were considered as a waxy shell [3] but this hypothesis has been abandoned.[4] Sabin, Smithburn, and Thomas,[5] working with rabbits, reported that these waxes neither produced necrosis nor damaged phagocytes in which they were contained. Thomas and Dessau,[6] on the other hand, recorded granulomata, including necrosis and giant cell formation, quite similar to the lesions resulting from living tubercle bacilli, following inoculation of mice. The waxes used by both groups of workers [5, 6] were unpurified, and derived from human and bovine tubercle bacilli as well as from leprosy bacilli.

Thomas [7] later reported production of granulomata containing many multinucleated giant cells by intraperitoneal administration in rabbits of phthiocerol, a higher alcohol derived from human tubercle bacilli. These bore no other resemblance to the granulomata of tuberculosis.

The materials used in the studies referred to were products of the extensive investigations of Anderson and his associates. More recently these investigators have isolated hydroxy acids by high temperature fractionation of the wax from various acid-fast organisms.[8] Through the courtesy of Dr. Anderson, it has been possible to study the reaction to several of these long chain fatty acids identified as mycolic acids. They include low and high melting point varieties derived from human and also from bovine and avian strains of tubercle bacilli as well as from leprosy bacilli (Table I).

Table I

1. Mycolic acid derived from human strain of tubercle bacillus	M.P. 55–56°
2. Mycolic acid derived from human strain of tubercle bacillus	M.P. 73–75°
3. Mycolic acid derived from leprosy bacillus	M.P. 62–63°
4. Mycolic acid derived from bovine tubercle bacillus	M.P. 56–58°
5. Mycolic acid derived from avian tubercle bacillus	M.P. 60–61°

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At 37°C. these mycolic acids are white amorphous powders that tend to clump so that homogeneous suspensions for animal inoculation are difficult to prepare, as has been noted previously with other waxes by Sabin, Smithburn, and Thomas [5] and by Thomas and Dessau.[6] By chilling and grinding in a homogenizer similar to that recommended by Corper and Cohn [9] and suspending as suggested by Anderson in 6 per cent gum arabic, a satisfactory preparation for prompt injection is secured. For intracutaneous injection 0.25 cc. of such suspension, containing 5 mg. of acid, was utilized and 4 cc., containing 20 mg. of acid, for intraperitoneal injection. For intravenous injection, 16 mg. of mycolic acid plus 2 to 3 cc. of distilled water were homogenized and further diluted with distilled water to which monoethanolamine (Eastman) had been added. The pH was then adjusted to 8.3 with normal hydrochloric acid and the volume adjusted so that each cc. contained 1 mg. of acid. At 37°C. this suspension became milky and contained particles of varying size as determined microscopically. With low-melting acids from human or avian tubercle bacilli, the particles were the size of a red blood cell; in the other preparations particles as large as polymorphonuclear leukocytes were seen. As a rule, 4 cc. of such a suspension was injected intravenously at intervals of 4 days until four treatments had been given. Several rabbits received an injection of 16 mg. for the study of changes during the first to fifth days.

Results

A. Intracutaneous Injection of Mycolic Acids

Gross observation, including measurement and classification of the lesions according to the degree of inflammation that resulted from intracutaneous injection, was carried out on five rabbits over a period of 45 days. Each of these animals was injected intracutaneously with three of the acids. Lesions induced by each of the acids could, therefore, be observed in three different rabbits.

The lesions resulting from the high-melting human mycolic acid and leprosinic acid were small and little evidence of inflammation was seen. They started to subside in all three rabbits about the 15th day. In contrast, the lesions following the injection of low-melting human mycolic acid and bovine mycolic acid became large and widespread on the 15th to 22nd day and in two of the rabbits the lesions were still distinct on the 39th day after injection. An initial inflammatory response followed the injection of avian mycolic acid. The lesions, however, were small on the 15th day and started to subside about the 22nd day. The point of injection of gum arabic alone as a control could not be identified 5 days after administration.

In a second series, lesions resulting from the intracutaneous injection of mycolic acid were excised on the 5th, 10th, 15th, 25th, and 40th to 45th day and studied microscopically. An initial stage of necrosis and of polymorphonuclear reaction was observed in every instance. Later the lesions were divisible into two groups. The injection of bovine mycolic acid and low-melting human mycolic acid was followed by formation of giant cells by the 5th day. These cells became large and their cytoplasm foamy by the 10th day. Deposit of calcium in both the lesions of low-melting human and bovine mycolic acid, and necrosis in the latter, 45 days after injection, furnished an additional differentiating factor.

In the lesions produced with the other three acids, giant cells were seen only on the 15th day. Slit-like cytoplasmic defects were present in them, apparently corresponding to the ingested mycolic acid. The fibrous tissue proliferation was moderate. Necrosis was absent in sections obtained after the 15th day and no calcium deposit was found.

B. 1. Intraperitoneal Injection of Mycolic Acids in Rabbits

Twenty-two rabbits were included in the group. Each received a single intraperitoneal injection of 20 mg. of acid suspended in gum arabic.

On the 15th day after injection, the low-melting human mycolic acid and the bovine mycolic acid distinguished themselves by producing large granulomatous lesions and extensive cellular disintegration (Figs. 1 and 3). The former were composed of multinucleated giant cells, numerous monocytes, and small round cells as well as polymorphonuclear leukocytes. Moderate to marked fibroblastic proliferation was noted. The giant cells were large, their nuclei numerous and the cytoplasm had assumed a foamy appearance due to the presence of many small vacuoles. At the 45th day, the lesions were still similar and showed the same cellular disintegration; numerous foamy monocytes and deposition of calcium were noted (Figs. 5 and 6).

Giant cells arranged in small groups, some monocytes and polymorphonuclear leukocytes characterized the lesions induced by high-melting human mycolic acid, leprosinic acid, and avian mycolic acid at the 15th day. Only a small amount of cellular disintegration was noted in the lesions resulting from the injection of leprosinic acid. The response to avian mycolic acid was minimal. Gum arabic itself induced giant cell formation of a slight degree. The giant cells contained a few nuclei and no vacuoles. This cellular reaction disappeared after the 15th day (Figs. 2 and 4).

On the 45th day the omenta of the rabbits injected with high-melting human mycolic acid were free of lesions save for a small amount of

fibrous tissue. The omenta of the rabbits that had received the avian mycolic acid revealed, in addition, an occasional group of two to four giant cells. The cellular disintegration found in the earlier lesions produced by leprosinic acid had disappeared, although the presence of the injected material within the giant cells could be demonstrated. The giant cells in this group were smaller, the nuclei less numerous, than in the first group, while the cytoplasmic defects were large and replaced much of the cell protoplasm.

The omental preparations obtained from this group of animals were of particular value in demonstrating that the vacuoles of the giant cells, as noted in sections secured by the usual technic, corresponded to the phagocytosed mycolic acid. Its identification was facilitated by acid fastness. Paraffin imbedding and its requisite fat solvents removed the mycolic acids. The frozen section method or imbedding in polyvinyl alcohols did not furnish sections of sufficient quality.

B. 2. Intraperitoneal Injection of Mycolic Acids in Guinea-Pigs

The structure of the lesions that resulted from intraperitoneal injection of the various mycolic acids in 20 guinea-pigs did not reveal differences as characteristic as those observed in rabbits. Granulomatous lesions composed of numerous multinucleated giant cells and vacuolated monocytes with a few fibroblasts interspersed were always present. The appearance of the giant cells did not vary with the different acids. Two weeks after injection, necrosis was found in all lesions except those induced with high-melting human mycolic acid.

Microscopic preparations obtained 6 weeks after the administration of the acids showed granulomatous lesions made up of numerous, round, multinucleated giant cells and some monocytes. The cytoplasmic defects in the case of the low-melting human mycolic acid were less numerous and smaller. A few fibroblasts were present between the giant cells. There was no evidence of cellular disintegration. The only lesion with well developed fibrous connective tissue resulted from avian mycolic acid.

C. Intravenous Injection of Mycolic Acid in Rabbits

This group comprised 33 animals. Each mycolic acid was injected as a suspension in water-ethanolamine. The latter compound alone produced no lesion.

A single intravenous injection of any of the mycolic acids caused, initially, intensive damage to pulmonary vessels as indicated by fibrinoid necrosis of the vessel walls and polymorphonuclear exudation throughout the vessel coat. No differentiating morphologic character-

istics for the various acids were found. Occasional giant cells with vacuolated cytoplasm were seen in the lumina of small arteries of the lung. Slight differences, depending upon the acid used, were found in the pulmonary lesions of rabbits sacrificed 24 hours after the last of four intravenous injections. The lesions of the low-melting human mycolic acid and of the bovine mycolic acid were more numerous, and consisted of polymorphonuclear leukocytes and monocytes surrounding giant cells that contained injected acid. The difference became more apparent on the 5th day, when the lesions induced by low-melting human mycolic and bovine mycolic acid were conspicuous both by their frequency and their granulomatous character (Fig. 7). Marked polymorphonuclear reaction was still present. The latter type of response was only slight with the other acids and absent with the high-melting human mycolic acid.

The giant cells with their contained low-melting human or bovine mycolic acid displayed several small vacuoles; those developed from the other acids exhibited large cytoplasmic defects (Figs. 8 and 9). The giant cells resulting from the injection of low-melting human or bovine mycolic acid (Fig. 7) contained many nuclei, those from the other acids fewer nuclei (Figs 8 and 9). These lesions were found exclusively in the lung.

At 15 and 45 days after injection, the frequency of the lesions in rabbits injected with low-melting human or bovine mycolic acid contrasted sharply with the rare ones after high-melting human mycolic, leprosinic or avian mycolic acid. The lesions of the two groups retained their different character. After low-melting human or bovine mycolic acid they were granuloma-like, containing large giant cells with numerous nuclei and vacuolated monocytes (Figs. 10 and 14). After the other three there were only isolated giant cells without further cellular reaction (Figs. 11, 12, 13, and 15).

At 45 days, small groups of vacuolated giant cells were found in the spleens, and occasionally in the livers, of animals injected with any of the mycolic acids.

Discussion

The mycolic acids, products obtained by fractionation of the waxes of tubercle bacilli and other acid-fast microorganisms, are largely responsible for the characteristic physicochemical properties of the waxes, including acid fastness. Lesions induced by them, therefore, might be expected to simulate those resulting from the waxes as described by Sabin, Smithburn, and Thomas.[5] This, in general, is the case. The initial polymorphonuclear reaction and secondary accumulation of

monocytes and of multinucleated giant cells, as observed in these investigations, correspond to lesions reported by Sabin *et al.*

The cellular response to the mycolic acids differs from that of the waxes inasmuch as all of the former result in initial and marked necrosis. This persists with low-melting human, and particularly with bovine, mycolic acid.

Although mycolic acids are distinguishable chemically only with difficulty, they differ in the reactions they excite in the rabbit. By these reactions these acids are roughly divisible into two groups. Those of the first group, including high-melting human and avian mycolic and leprosinic acid, are only slowly fragmented into small particles and cause initial but nonpersistent necrosis. The subsequent lesions are small, consisting largely of giant cells that contain few vacuoles and are surrounded by a minimal proliferative reaction.

Acids of the second group, low-melting human and bovine mycolic acid, rapidly resolve into finer particles and bring about extensive and persistent necrosis. The lesions retain their character for several weeks.

No known chemical difference among various types of tubercle bacilli has shed any light so far upon the problem of native resistance. Variation in cellular response to the mycolic acids may be related to differences in susceptibility of animals to the strain of tubercle bacilli from which the acid is derived. This correlation is, however, incomplete. The rabbit is known to be susceptible to both bovine and avian tubercle bacilli. The mycolic acid recovered from the bovine strain is more effective in producing long-standing necrosis and extensive cellular response. Avian mycolic acid, however, in its biologic result, is not unlike that of the leprosy bacillus to which this animal is refractory. Reciprocally, low-melting mycolic acid from the human tubercle bacillus produces lesions similar to those caused by bovine mycolic acids although the rabbit is not susceptible to the latter mycobacterium.

The mycolic acids as constituents of the tubercle bacilli may be assumed, therefore, to contribute to the persistence of lesions induced by this microorganism, but there is no evidence that they are related to the elective pathogenicity of different types of tubercle bacilli.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

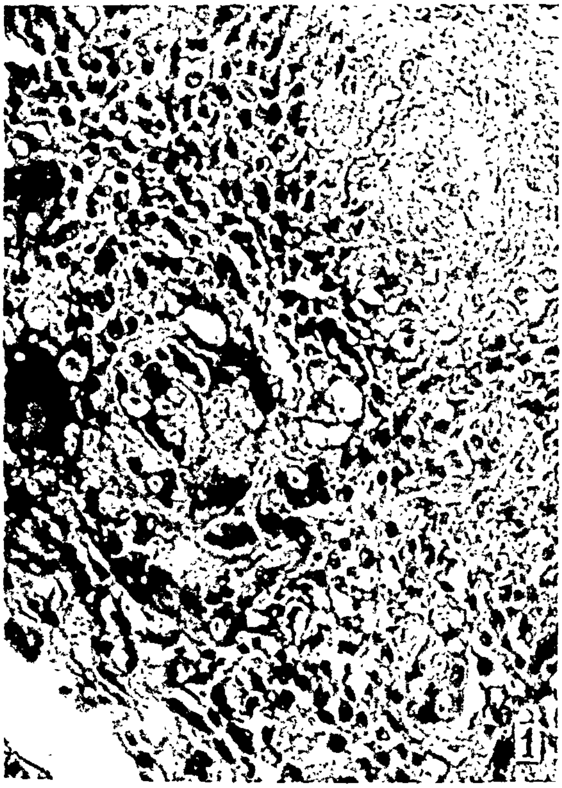
PLATE 179

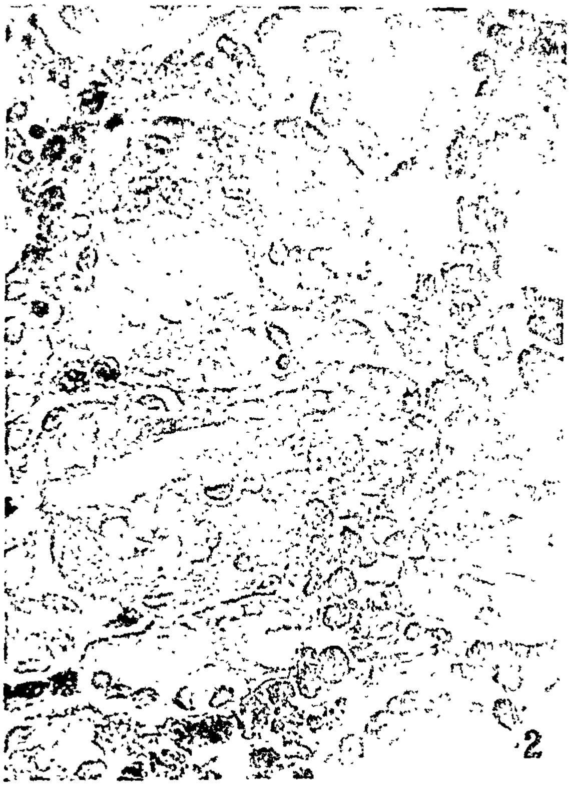
FIG. 1. Rabbit 902. Omentum on the 15th day after the intraperitoneal injection of a low-melting human mycolic acid. Hematoxylin and eosin stain. × 400.

FIG. 2. Rabbit 909. Omentum on the 15th day after the intraperitoneal injection of a high-melting human mycolic acid. Hematoxylin and eosin stain. × 500.

FIG. 3. Rabbit 894. Omentum on the 15th day after the intraperitoneal injection of bovine mycolic acid. Hematoxylin and eosin stain. × 225.

FIG. 4. Rabbit 898. Omentum on the 15th day after the intraperitoneal injection of leprosinic acid. Hematoxylin and eosin stain. × 400.





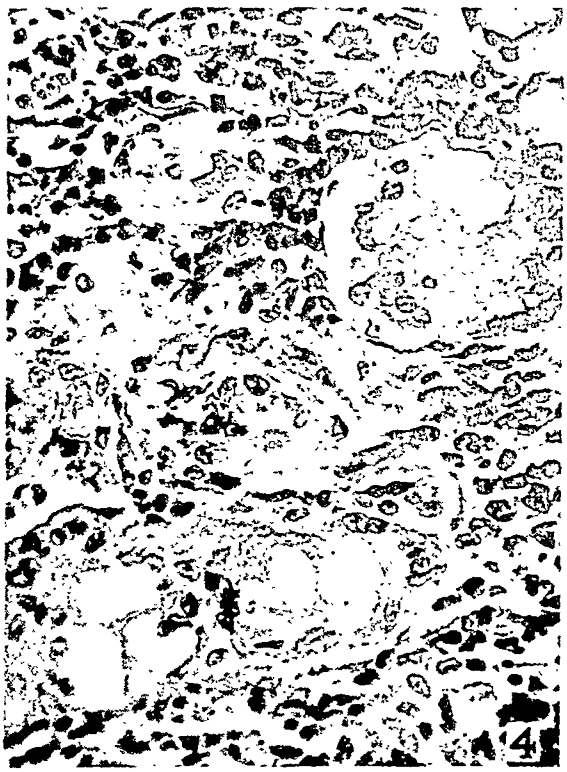


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PLATE 180

FIG. 5. Rabbit 899. Omentum on the 45th day after the intraperitoneal injection of bovine mycolic acid. Hematoxylin and eosin stain. × 285.

FIG. 6. Rabbit 899. Omentum on the 45th day after the intraperitoneal injection of bovine mycolic acid. Hematoxylin and eosin stain. × 235.

FIG. 7. Rabbit 63. Lung on the 5th day after the intravenous injection of a low-melting human mycolic acid. Hematoxylin and eosin stain. × 325.

FIG. 8. Rabbit 61. Lung on the 5th day after the intravenous injection of leprosinic acid. Hematoxylin and eosin stain. × 325.

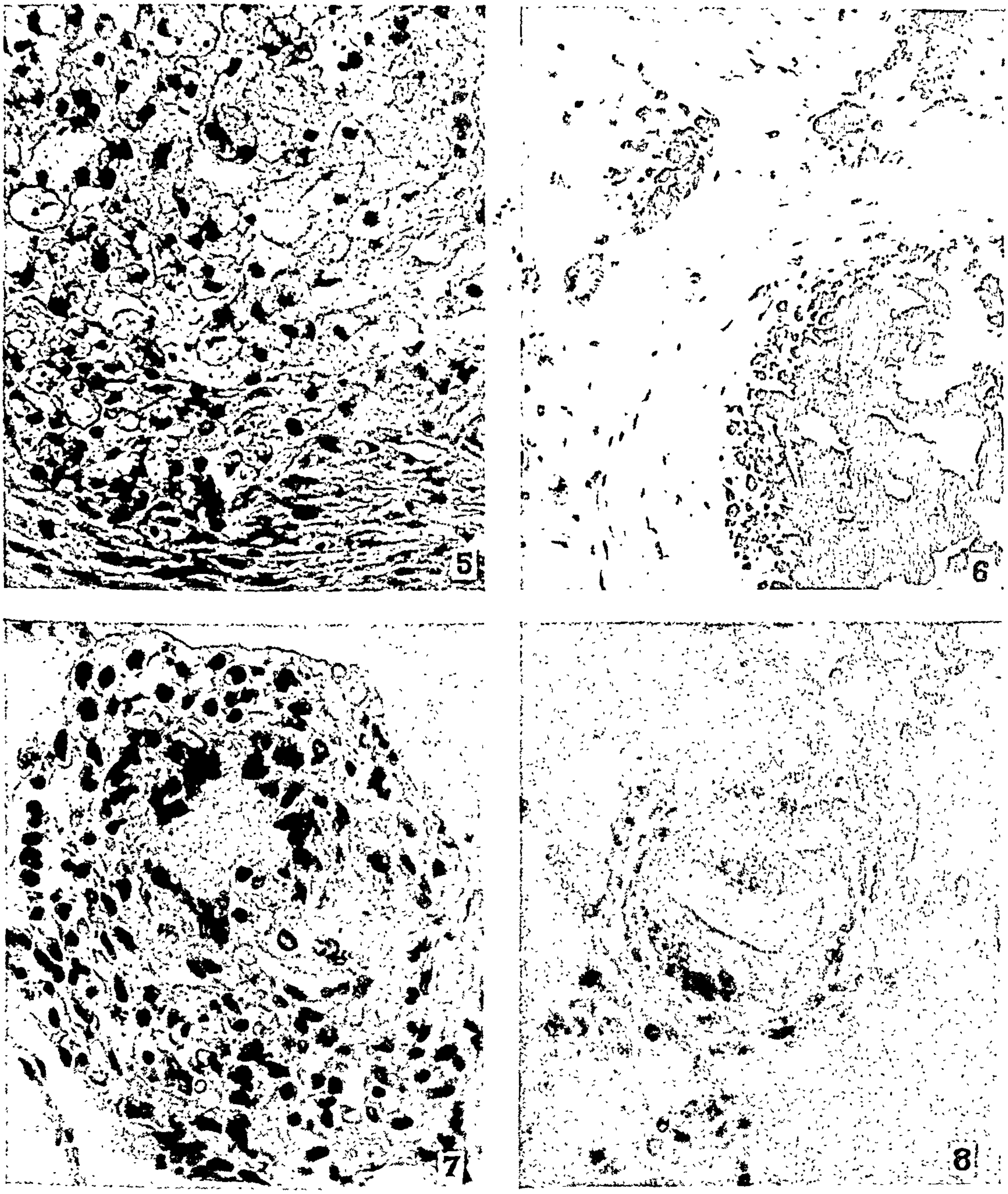

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PLATE 181

FIG. 9. Rabbit 66. Lung on the 5th day after intravenous injection of avian mycolic acid. Hematoxylin and eosin stain. × 700.

FIG. 10. Rabbit 13. Lung on the 15th day after the intravenous injection of a low-melting human mycolic acid. Hematoxylin and eosin stain. × 475.

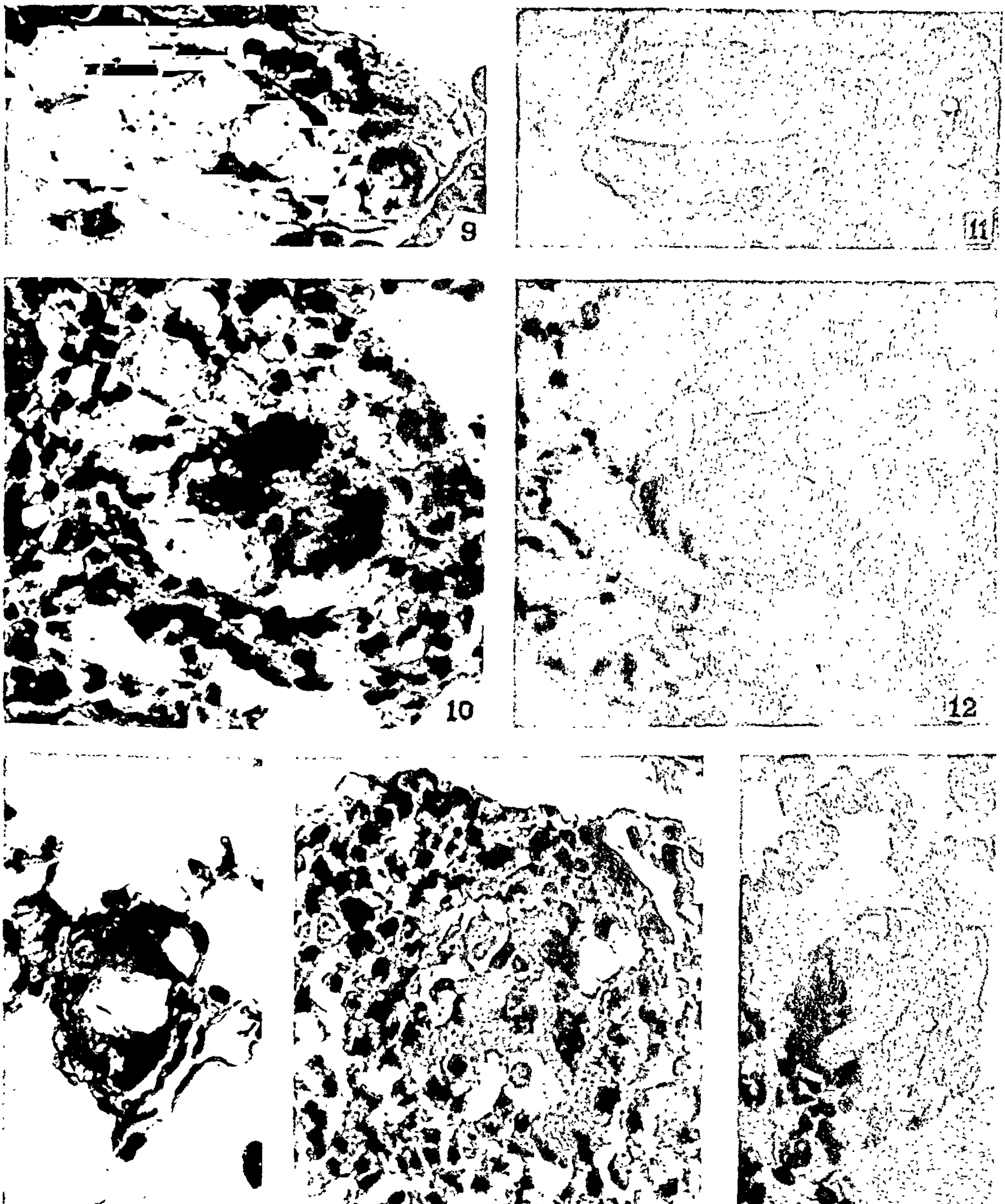
FIG. 11. Rabbit 16. Lung on the 15th day after the intravenous injection of a high-melting human mycolic acid. Hematoxylin and eosin stain. × 325.

FIG. 12. Rabbit 30. Lung on the 15th day after the intravenous injection of leprosinic acid. Hematoxylin and eosin stain. × 325.

FIG. 13. Rabbit 22. Lung on the 45th day after the intravenous injection of a high-melting human mycolic acid. Hematoxylin and eosin stain. × 475.

FIG. 14. Rabbit 29. Lung on the 45th day after the intravenous injection of bovine mycolic acid. Hematoxylin and eosin stain. × 485.

FIG. 15. Rabbit 18. Lung on the 45th day after the intravenous injection of avian mycolic acid. Hematoxylin and eosin stain. × 400.



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Cellular Reactions to Mycolic Acids

EXPERIMENTAL STUDIES IN CARDIOVASCULAR PATHOLOGY

XI. Thesaurosis and Atheromatosis Produced in Dogs by the Repeated Intravenous Injection of Solutions of Sodium Cellulose Glycollate *

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It was shown in previous investigations that colloidal aqueous solutions of methyl cellulose are effective substitutes for plasma and elicit, when introduced in highly excessive amounts over prolonged periods, symptoms of thesaurosis and atheromatosis (Hueper,[1, 2] Hueper and Ichniowski [3]). When a new water-soluble derivative of cellulose, the sodium salt of cellulose glycollic acid, became available, a toxicopathologic study of this substance appeared to be of interest.

Experimental Procedure

Sodium cellulose glycollate (Collocel S), which is manufactured by the Dow Chemical Co. in three grades of viscosity, is a cellulose ether with a carbomethoxyl ($-OCH_2COOH$) radical. As the degree of substitution is relatively low, there is a predominance of free hydroxyl groups. Collocel S of high viscosity is a white, odorless, granular powder readily soluble in cold and boiling water, giving a slightly turbid, grayish white solution of neutral reaction. After being filtered through Hyflo-Supercel † and filterpaper, a slight haziness remains. According to the statements of the manufacturer, Collocel is compatible with the majority of the acid dyes, but with very few of the basic dyes. Solutions of Collocel do not form foam upon shaking. A 0.25 per cent aqueous solution has a colloidal osmotic pressure of approximately 140 mm. of water.

A 0.25 per cent solution of the high viscosity grade of Collocel in 1 per cent sodium chloride solution was used in the experiments. It has a viscosity of 1.8 at 18°C. This solution was injected into the jugular veins of 5 dogs, 3 of which weighed 8 to 8.5 kg., 1 weighed 10.3 kg., and the fifth, 14.5 kg. Four of the dogs were from 1.5 to 3.5 years old, while the fifth was 6 years old. For the study of the immediate hematic reactions 4 dogs received a single dose of 40 cc. Blood was withdrawn 5, 15, 30, 60, 120, 240 minutes, and 24 hours after injection. The following blood constituents were determined: Amount of hemoglobin, number of erythrocytes and leukocytes, differential count, sedimentation rate volume of packed blood cells, plasma viscosity. Additional daily injec-

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† Hyflo Supercel is a preparation of purified diatomaceous earth, obtained from the Dicalite Co., 120 Wall Street, New York, N.Y.

tions of the same dose were made in 2 dogs after an interval of 7 days. After having been continued for 3 months, the daily dose was increased to 100 cc. for an additional period of 4 weeks, when the surviving dog was sacrificed after receiving a total of 4800 cc. of the solution containing 12 gm. of sodium cellulose glycollate. The second dog belonging to this set died after having received 7 injections of 100 cc. each.

The other 2 dogs used in the studies of the immediate reactions as well as a fifth dog received, beginning 24 hours after the first injection, daily intravenous administrations of the Collocel solution. The daily dose was 40 cc. for the first month, was increased to 75 cc. during the second month, and to 150 cc. during the third month. One of these 3 dogs was sacrificed after 16 days, the other 2 after 2.5 months, after having received a total of 6,485 cc. of the solution containing 16.25 gm. of sodium cellulose glycollate.

Hematic Reactions after a Single Intravenous Injection of Collocel

The erythrocytes increased from original values, fluctuating between 7.0 and 7.4 million, to values of 8.5 to 8.8 million 5 minutes after the injection of Collocel. This movement was followed by a gradual drop to approximately original levels during the following 2 hours when a second elevation of the erythrocytes was seen in the tests made at the 2 and 4 hour periods. The erythrocytes ranged then from 8.0 to 8.7 millions. Approximately original values prevailed again at the 24-hour test. The hemoglobin values underwent less appreciable changes and showed more often a tendency to decline than to rise. There occurred in all instances a moderate to marked leukopenia 5 minutes after injection, which lasted from 5 minutes to 3 hours in the different animals, but was followed in only one instance by a secondary leukocytosis. The differential counts revealed a mild shift toward the left coinciding with the leukopenic phase, but this development remained, in general, within normal limits. There were no appreciable or significant fluctuations in the viscosity of the plasma or in the sedimentation rate of the erythrocytes during the first 24 hours after injection. The platelets were extraordinarily large and of "parasite-like" appearance due to the frequent presence of flagella-like processes.

Hematic Reactions after Repeated Intravenous Injection of Collocel

'he repeated intravenous injection of Collocel solutions was well tolerated. There were no temperature reactions, but immediately after each injection a transitory leukopenia developed. During the third and

fourth months the amount of hemoglobin gradually decreased to approximately 75 to 80 per cent of the original level. No significant abnormalities were found in the number of erythrocytes and leukocytes, nor in the differential count and the viscosity of the plasma. The sedimentation rate, on the other hand, increased progressively during the last 2 months, when it fluctuated between 30 and 40 mm. (Wintrobe-Landsberg tube).

PATHOLOGICAL OBSERVATIONS

The post-mortem examination of the 5 dogs did not reveal any appreciable macroscopic abnormalities of the internal organs. Particularly, the liver and spleen were not significantly enlarged nor did they deviate in consistency and color from normal organs.

Histologic studies were made of the lungs, heart, thyroid, parathyroid, liver, spleen, pancreas, intestine, adrenal, kidney, testis, aorta and its large branches. The aorta was cut for this purpose into 15 to 20 rings, while 2 or 3 rings each were cut from the carotid, brachial, and iliac arteries.

The dog which was sacrificed on the 16th day of the experiment had histologically normal organs with the exception of the presence of scanty amount of bluish homogeneous deposits found intracellularly and extracellularly in the splenic pulp in sections stained with hematoxylin and eosin.

The histologic findings in the other 4 dogs were essentially uniform. The ascending aorta showed occasional, small, localized proliferations of endothelial cells forming bundles running parallel to the longitudinal axis, and large fibroblastic-mononuclear edematous cushions containing numerous multinucleated giant cells. Foam cells filled with homogeneous matter, staining a moderately dark blue, were present in parts of these cushions and, in a few places, beneath the endothelial lining. In some segments of the thoracic aorta scattered small elevations of endothelial foam cells containing bluish matter as well as extracellular bluish material occurred beneath an endothelial lining showing cellular crowding. Such foci were observed at the orifices of branches, where they were found within, or extended into, the funnel-like first part of the branching vessel (Fig. 1). Areas exhibiting foam-cellular swelling of the endothelial lining were scattered here and there (Fig. 2). Similar foam cell lesions were encountered in several vasa vasorum located in the media or adventitia where foam cells were sometimes seen in the perivascular spaces of these vessels. In the lumen of one vas vasis a bluish mass was split up by numerous slender cells (Fig. 3). The inner media was often edematous, while fibro-hyaline foci were occasionally

seen in the outer media. Similar foam-cellular lesions occurred rarely in the large arterial branches of the aorta of only one animal. Atheromatous lesions in general were small and infrequent.

The Kupffer cells of the liver were balloon-like, swollen and proliferating, and contained a blue-stained homogeneous cytoplasm, while the nucleus was crescent-shaped and pushed against the cellular wall. The liver cells were intact. The pulp of the spleen contained many bluish staining foam cells as well as strands of a homogeneous extracellular bluish material. Mononuclear giant cells in a moderate number were present in the spleen of one dog (Fig. 5). The renal glomeruli contained scattered groups of swollen endothelial cells with a bluish content. Similar foam cells were occasionally seen in groups in the interstitial tissue (Fig. 4), particularly in the perivascular areas. Bluish staining casts were found in the tubular lumina of the medulla.

All other organs were without any significant changes.

Comment

The hematic and anatomic changes observed in dogs after the intravenous injection of solutions of sodium cellulose glycollate resemble in many respects those seen in dogs and rabbits after the intravenous introduction of colloidal solutions of other macromolecular carbohydrates such as methyl cellulose, polyvinyl alcohol, pectin, gum arabic (Hueper[2]), inasmuch as there appeared a transitory leukopenia, a decrease in hemoglobin, an increase in the sedimentation rate of the erythrocytes, a deposition of the injected matter in the reticulo-endothelial cells of the liver and spleen and in the glomerular endothelial cells and interstitial cells of the kidney, and the formation of atheromatous deposits in the aorta and its branches. It is remarkable, on the other hand, that these reactions remained within relatively moderate limits when compared with those seen in connection with the other above-mentioned substances, especially the methyl celluloses, if proper consideration is given to the total amount of colloidal material injected. The reason for this discrepancy must remain uncertain as no data are available on the molecular size of this compound and on the rôle which the glycollic acid part of the molecule might have played in its colloidal dispersion, in the colloidal stability of the plasma, and the permeability of the filtration membrane. The presence of bluish staining casts in the tubular lumina of the kidney indicates that the injected material is excreted through the kidney.

In contrast to methyl cellulose, sodium cellulose glycollate is not taken up by the liver cells, but only by the stellate cells. The atheromatous formation evidently occurs, as with other colloidal and macro-

molecular atheromatogenic agents, by the intracellular resorption of this material from the plasma into the endothelial cells and by its extracellular infiltration into the subendothelial spaces of the aorta and its large branches as well as of their vasa vasorum. The localization of these deposits in the region of the orifices of branches furnishes additional evidence supporting the contention that the normal turbulence of the blood, and vibratory lability of the plasma colloids in certain areas produced thereby, represent important factors controlling the distribution of atheromas in the vascular tree (Hueper [4]).

While the reported evidence is not unfavorable as to the suitability of solutions of sodium cellulose glycollate as a plasma substitute, blood pressure measurements made on anesthetized dogs injected with solutions of this substance showed that it exerts a definite depressor effect which obviates its use as a therapeutic agent in the treatment of shock.

Conclusions

Solutions of sodium cellulose glycollate, injected intravenously, elicit in dogs a transitory leukopenia and, upon repeated introduction, a decrease in hemoglobin and an increase in the sedimentation rate.

Sodium cellulose glycollate is stored in the Kupffer cells, the reticulum cells of the spleen, the endothelial cells of the renal glomeruli, and as atheromatous deposits in the aorta and its large branches.

The anatomical character and the distribution of the atheromatous lesions support the concept that in the genesis of these changes the macromolecular colloidal matter present in the plasma is taken up by the endothelial cells, which are transformed thereby into foam cells, and infiltrates extracellularly into the subendothelial spaces in which normally a turbulence of the blood exists and where thereby a vibratory lability of the plasma colloids is produced.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

Plate 182

Fig. 1. Broad-based endothelial proliferation at the orifice of a small branch of the aorta. Hematoxylin and eosin stain. × 180.

Fig. 2. Endothelial foam cells coating the wall of the aorta. Hematoxylin and eosin stain. × 270.

Fig. 3. Foam-cellular proliferation in and around vasa vasorum of the aortic wall. Hematoxylin and eosin stain. × 270.

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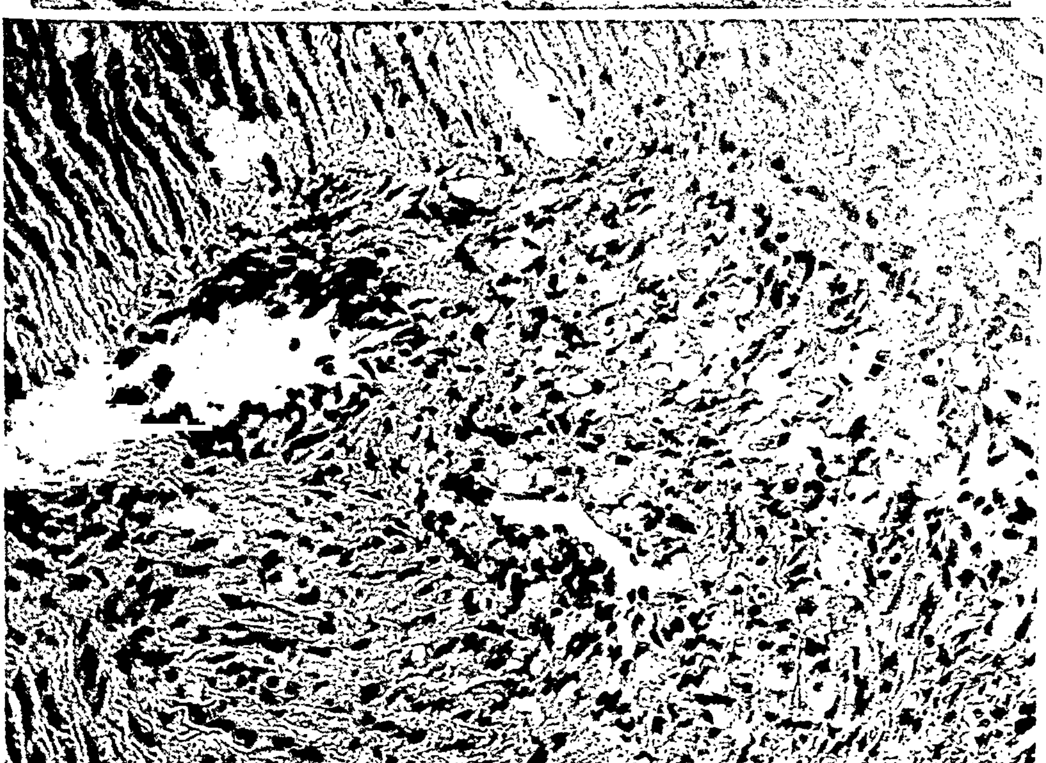

Hueper

Sodium Cellulose Glycollate

PLATE 183

FIG. 4. Capillary loops of a glomerulus obliterated by swollen endothelial foam cells. Hematoxylin and eosin stain. × 360.

FIG. 5. Accumulations of reticulocytic foam cells in the pulp of the spleen. Hematoxylin and eosin stain. × 270.

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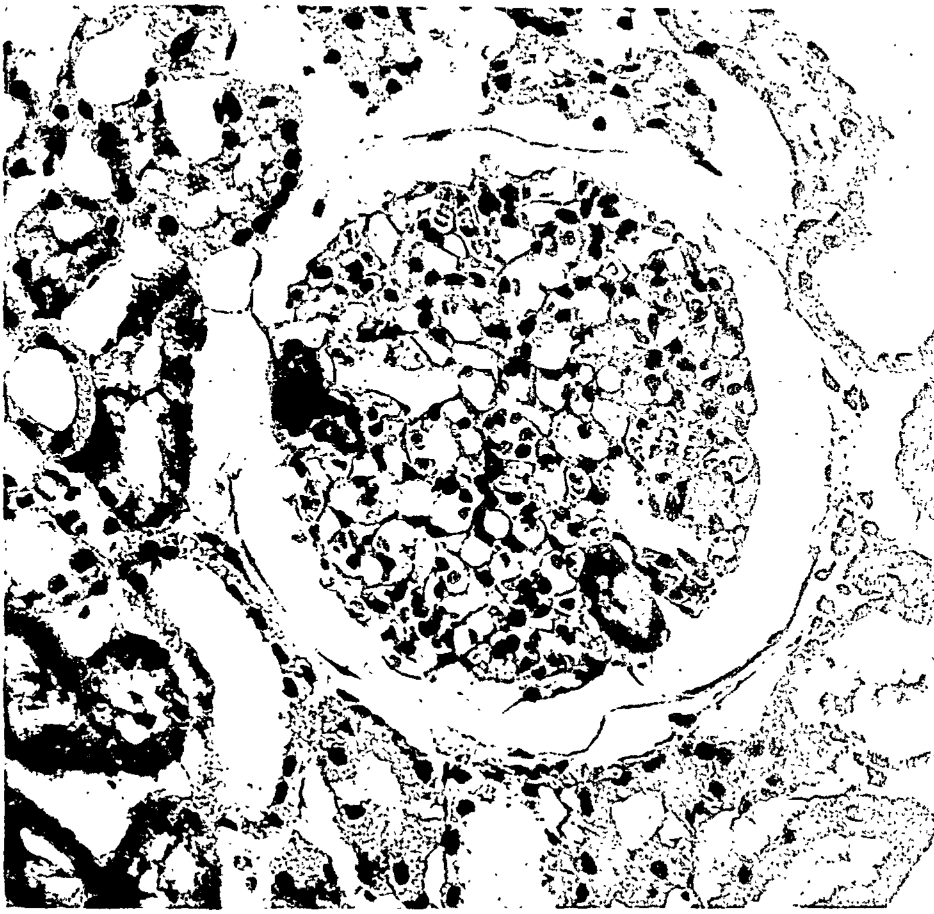

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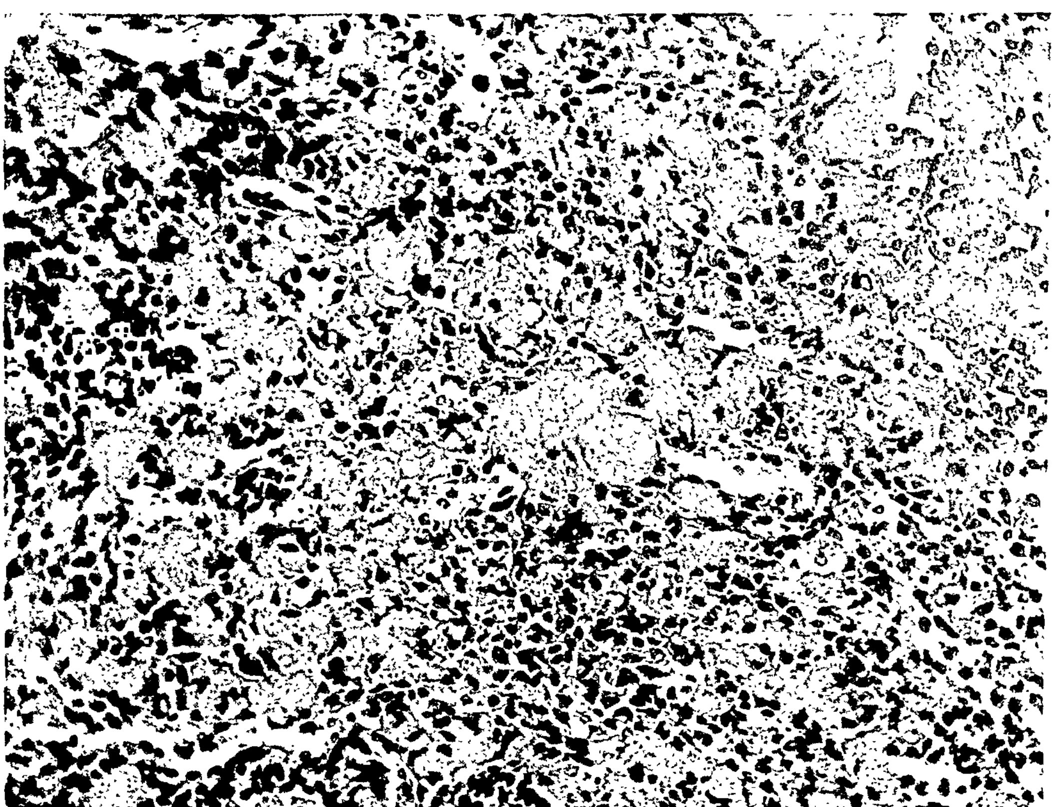

Hueper Sodium Cellulose Glycollate

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THE RACIAL DISTRIBUTION OF NEPHRITIS AND HYPERTENSION IN PANAMA *

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In Panama a large scale natural experiment on the pathogenesis of human hypertension awaits scientific interpretation. The studies of Kean [1] and of Marvin and Smith [2] have demonstrated the presence of fairly distinct racial groups, living in contiguity and subjected to similar environmental factors, in which there is a striking difference in the incidence of hypertension. The native Panamanians originally were Indians, but in the past 300 years there has been added to this stock the blood of Spaniards and other Europeans together with their Negro slaves. This apparently composite ethnologic group is actually fairly clearly defined in language, customs, and appearance. Relatively pure-blooded Negroes were imported to Panama from the West Indian Islands for construction work on the Canal 30 to 40 years ago and with their descendants they form another rather distinct group. These racial groups were defined by Kean [1] as follows: "A 'Panamanian' is one born in Panama whose parents were both born in Panama," and "a 'West Indian' is a Negro who was either born in the West Indies of West Indian parentage or whose parents both were born in the West Indies." A third racial group is made-up of Caucasians, most of whom are United States citizens.

In examining 1328 candidates for employment with the Panama Canal, Kean [1] found that hypertension was seven times as common in the West Indians as in the Panamanians; this difference was especially marked in the younger age groups in which the ratio of Negro to Panamanian hypertensive patients ranged as high as 16 to 1. In a group of almost 2,000 pregnant females he found hypertension to be five times as frequent in the West Indians as in the Panamanians. In over 2,000 consecutive hospital admissions Marvin and Smith [2] found that hypertension was about eight times as common in the West Indians as in the Panamanians.

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Phillips[3] found a high incidence of hypertension in Negroes in Jamaica. It is the consensus of studies[4-6] of the racial incidence of hypertension in the United States that American Negroes have about twice as much hypertension as whites. Many factors have been considered in attempting to explain this difference. Heredity has been discounted because of Donnison's[7] report of the relatively low blood pressure found in African Negroes living under primitive conditions. These authors suggested that hypertension in the Negro may be caused in some way by adjustment to a new civilization and a new environment.

Shattuck[8,9] has reported that the Indians of Guatemala and of Yucatan have relative hypotension and that blood pressure in the mestizo or mixed racial groups was somewhat higher. Kean,[10] in a survey of the relatively isolated Cuna Indians living on the San Blas Islands off the coast of Panama, observed that the average blood pressure of 407 adult Indians was 105 mm. of Hg systolic and 69 diastolic; not a single case of hypertension was found.

The generally recognized correlation between hypertension and nephritis suggested that an analysis of the racial distribution of nephritis in Panama might contribute to our understanding of the problem.

Material and Methods

At the Board of Health Laboratory autopsies are performed on a large percentage of the patients dying in the hospitals of the Canal Zone and on coroner's cases and those of the police from the Canal Zone and Republic of Panama. On most of these patients fairly adequate clinical records are available. All autopsies are complete, and in all instances slides and tissue blocks have been filed and are available for examination.

In this study 800 consecutive autopsy records from 1939 to 1942 were reviewed. All cases on which there were insufficient clinical data were first eliminated, leaving a group of 498 comparable cases which in the subsequent discussion will be referred to as the *total group*. This total group was then subdivided according to race as follows: West Indian Negroes, 266; Panamanians, 77; whites, 135; miscellaneous, 20. From the total group all cases showing any sign of hypertension or nephritis were selected for special study according to the following criteria. All the blood pressure readings of each patient were averaged and if the mean level was over 140/90, or if there was any evidence of acute or chronic nephritis in gross and microscopic examinations of the kidneys, the cases were included in the *study group*.

Slides of the kidneys of the 193 cases in the study group were personally reviewed without referring to the clinical data, and the nephritic changes were classified according to the outline in Table I. This outline

is based upon pathologic descriptions in standard textbooks, except for pyelonephritis. The cases of pyelonephritis were classified according

TABLE I

Histologic Criteria for the Diagnosis of Nephritis

1. Glomerulonephritis
   A. Acute and subacute
      (1) Proliferative reaction in capillary tufts with hyaline material in capillary walls
      (2) Polymorphonuclear leukocytic infiltration of capillary tufts
      (3) Cellular and albuminous exudate in Bowman's spaces
      (4) Epithelial crescents
      (5) Tubular degeneration and casts (may show picture of "lipoid nephrosis")
   B. Chronic
      (1) Glomerular hyalinization and fibrosis
      (2) Epithelial crescents—occasionally
      (3) Atrophy of tubules with compensatory hypertrophy and dilatation of others
      (4) Interstitial fibrosis and small round cell reaction
      (5) Vascular sclerosis
2. Pyelonephritis
   A. Acute
      (1) Focal abscesses with polymorphonuclear leukocytic reaction
      (2) Leukocytic casts in tubules
      (3) Localized or diffuse acute inflammatory reaction of interstitial tissues with predominantly perivascular pattern
      (4) Pelvic and peripelvic inflammatory reaction
      (5) Degeneration of tubular epithelium
   B. Active chronic
      (1) Combination of A and C
   C. Healed
      (1) Pyelonephritic scars consisting of localized areas of small round cell reaction and fibrosis frequently adjacent to vessels of cortex or medulla
      (2) Peripelvic chronic inflammatory reaction
      (3) Periglomerular fibrosis
      (4) Tubular atrophy with colloid casts
      (5) Arterial and arteriolar sclerosis usually most marked in and near pyelonephritic scars
3. Nephrosclerosis
   A. Arterial
      (1) Medial thickening and intimal proliferation of arterial walls with or without cholesterol deposits
      (2) Wedge-shaped scars
   B. Arteriolar
      (1) Subintimal hyalinization of afferent arterioles with obvious reduction of lumina
      (2) Intimal proliferation and medial thickening of small arteries
      (3) Occasionally necrotizing arteriolitis and proliferative endarteritis of "malignant nephrosclerosis"
      (4) Ischemic glomerular fibrosis or hyalinization
      (5) Tubular atrophy
      (6) Diffuse fibrosis and slight lymphocytic reaction
4. Unclassified chronic nephritis—combination of 1, 2, and 3, defying classification

to the pathologic criteria proposed by Longcope,[11] Weiss and Parker,[12,13] Kimmelstiel and Wilson,[14] Mallory, Crane, and Edwards,[15] and Mansfield, Mallory, and Ellis.[16] In the nephrosclerotic group, sclerotic changes of only arterioles and smaller arteries were recorded. Since the gross specimens were not available it was impossible to conduct a comparative study of the larger arteries. Considerable difficulty was experienced in classifying a few cases of severely contracted kidney and also with some cases which showed slight nephrosclerotic changes but also met some of the criteria of healed pyelonephritis. All of these doubtful cases were grouped under unclassified nephritis.

No attempt was made to classify the cases on the basis of benign or malignant hypertension. In the histologic survey, however, several cases were noted that showed the necrotizing arteriolitis or proliferative endarteritis which are thought to be associated with the syndrome of malignant hypertension. These changes were observed in cases of severe pyelonephritis as well as nephrosclerosis.

Nephrosis was not included in this classification; first, because there is some doubt about the occurrence of lipoid nephrosis as a clinical and pathologic entity, and second, because the only cases of nephrosis encountered were due to acute poisoning and were not included in the study group.

Data

Racial Distribution of Nephritis

In Table II the 193 cases in the study group are classified according to race and type of nephritic change. When the original classification was made the severity of the pathologic change was graded from 1 to 4 plus. These figures were too cumbersome for presentation and the essential information was obtained by grouping grades 1 and 2 under the mild cases and grades 3 and 4 under the severe cases.

In the entire study group arteriolosclerosis is comparatively common, pyelonephritis is even more common, and glomerulonephritis is relatively rare.

West Indian Negroes. Because most of the cases of nephritis occurred in West Indians, the relative incidence of nephritis in this group does not differ significantly from that of the entire study group. Cases of nephrosclerosis and pyelonephritis are almost equally divided and together constitute 79 per cent of the total cases of nephritis in Negroes. A striking difference between these two types of nephritis is seen: 48 per cent of the cases of pyelonephritis were classified as severe, whereas only 17.6 per cent of the cases of nephrosclerosis fell into this group. It may be that the severity of the nephritis in these groups would have

been more nearly balanced if it had been possible to classify more accurately the cases of unclassified nephritis which constituted 19 per cent of the total nephritis in Negroes. Glomerulonephritis was present in only 2 per cent of the cases.

Panamanians. In spite of the fact that the total number of cases of nephritis in Panamanians is so small, it seems significant that most

TABLE II

Racial Distribution of Renal Lesions in Study Group

	Glomerulo-nephritis	Pyelonephritis				Nephro-sclerosis	Unclas-sified	Negative
		Total	Healed	Active chronic	Acute			
All races (193 cases)								
Severe	1	33	(20)	(11)	(2)	10	3	
Mild	3	39	(34)	(3)	(2)	53	33	
Total	4	72	(54)	(14)	(4)	63	36	18
Percentage	2	41	(31)	(8)	(2)	36	21	
Negroes (143 cases)								
Severe	1	25	(18)	(6)	(1)	9	2	
Mild	1	27	(24)	(3)		42	24	
Total	2	52	(42)	(9)	(1)	51	26	12
Percentage	2	40	(32)	(7)	(1)	39	19	
Panamanians (12 cases)								
Severe		2	(1)	(1)			1	
Mild	1	5	(4)		(1)	2	1	
Total	1	7	(5)	(1)	(1)	2	2	
Percentage	8	58	(42)	(8)	(8)	17	17	
Whites (34 cases)								
Severe		4	(1)	(2)	(1)	1	1	
Mild	1	7	(6)		(1)	8	6	
Total	1	11	(7)	(2)	(2)	9	7	6
Percentage	4	39	(25)	(7)	(7)	32	25	

of them are of pyelonephritis. When the 12 cases are individually analyzed it is found that 3 cases of pyelonephritis and 1 case of unclassified nephritis occurred in lepers, in whom nephritis is common, and that 2 more patients with pyelonephritis died of tuberculosis.

Whites. The most significant point to be observed is that nephritis in whites in Panama more closely resembles the general distribution of nephritis in Panama than it does the distribution of nephritis among whites in temperate zones. Only 1 mild case of acute glomerulonephritis was found in 135 autopsies. No evidence of nephritis was observed in 18 per cent of the white hypertensive patients.

Sex Distribution of Nephritis

In Table III the distribution of the cases according to sex is shown. The general autopsy rates at the Board of Health Laboratory show a preponderance of males over females of 3.3 to 1 in West Indians, 8 to 1 in whites, and 1.5 to 1 in Panamanians. When considered in relation to these rates, the proportion of nephritis in males and females is approximately what might be expected in the West Indians and whites. The high incidence of nephritis in Panamanian males is due almost entirely to the high incidence of pyelonephritis in this group. The percentage incidence of cases of healed and active chronic pyelonephritis in the nephritic groups that were large enough to permit analysis was: black males, 37.6 per cent; black females, 29.4 per cent; and white males, 21.4 per cent. The incidence of nephrosclerosis in the same groups was: black males, 32 per cent; black females, 47 per cent; and white males, 30.4 per cent.

TABLE III

Distribution of Nephritis According to Sex

	Negroes		Panamanians		Whites	
	Male	Female	Male	Female	Male	Female
Glomerulonephritis	1	1		1	1	
Pyelonephritis						
Total	42	10	6	1	8	3
Healed	35	7	4	1	5	2
Active chronic	6	3	1		1	1
Acute	1		1		2	
Nephrosclerosis	35	16	1	1	7	2
Unclassified	20	6	2		7	
Negative	11	1			5	1
Total	109	34	9	3	28	6

Age Distribution

The average ages of the various races in the study group were: Negroes, 56.8 years; Panamanians, 47.3 years; and whites, 55.2 years. Although the Panamanians are apparently younger than the other racial groups, there are so few cases that the standard error of their average age is $\pm 2 \times 7.87$ which brings this difference well within the range of a chance occurrence.

Incidence of Nephritis in Total Group

Table IV and Text-Figure 1 contain the most significant data presented in this paper. The percentage incidence of the various types of nephritis in the total group and the average blood pressures for each group are shown. The incidence of each type of nephritis within a

racial group is shown best in Table II, while in Table IV the incidence of a specific type of nephritis in one race can be more readily compared with its incidence in the other racial groups.

Among the West Indians autopsied, 49.3 per cent had some form of nephritis in contrast to 15.6 per cent of the Panamanians and 20.7 per

TABLE IV

Nephritis and Blood Pressure in Total Autopsy Group

	Negroes (266 cases)			Panamanians (77 cases)			Whites (135 cases)		
	Cases	Per-centage	Blood pressure	Cases	Per-centage	Blood pressure	Cases	Per-centage	Blood pressure
Glomerulo-nephritis	2	0.7	150/85	1	1.3		1	0.7	140/90
Pyelonephritis	52	19.6		7	9.1	—	11	8.2	
Healed	(42)	(15.8)	178/107	(5)	(6.5)	136/86	(7)	(5.2)	160/92
Active chronic	(9)	(3.4)	165/94	(1)	(1.3)	151/84	(2)	(1.5)	140/83
Acute	(1)	(0.4)	165/80	(1)	(1.3)	117/70	(2)	(1.5)	150/85
Nephrosclerosis	51	19.2	180/107	2	2.6	145/100	9	6.7	168/108
Unclassified	26	9.8	172/101	2	2.6	133/96	7	5.2	155/98
Total nephritis	131	49.3	176/104	12	15.6	136/89	28	20.8	159/98
Negative	12	4.5	156/95	0			6	4.4	155/96
Total	143	53.8	174/103	12	15.6	136/89	34	25.2	158/97

Standard Errors of Differences in Percentages

Total Nephritis:
- Negro to Panamanian $\pm 2 \times 5.15$
- Negro to White $\pm 2 \times 4.64$
- White to Panamanian $\pm 2 \times 5.42$

Pyelonephritis:
- Negro to Panamanian $\pm 2 \times 4.02$
- Negro to White $\pm 2 \times 3.37$

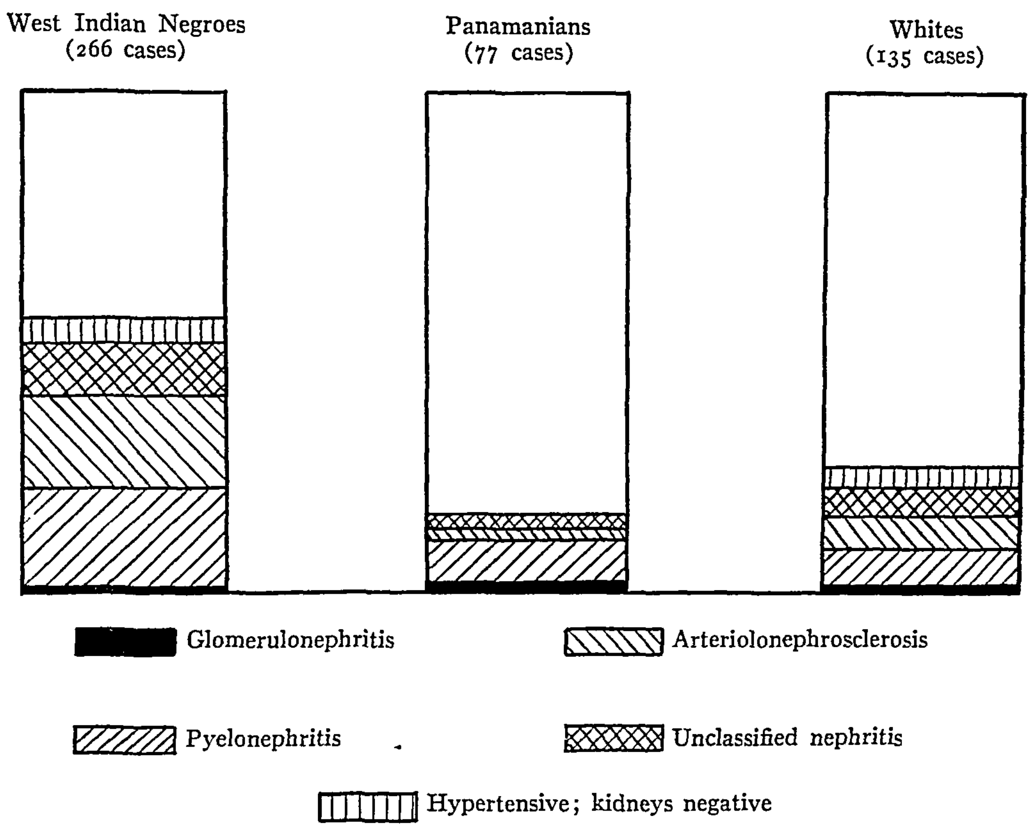
Nephrosclerosis:
- Negro to Panamanian $\pm 2 \times 3.02$
- Negro to White $\pm 2 \times 3.24$

cent of the whites. This marked difference is evident in all of the specific types of nephritis except glomerulonephritis, which is equally low in all groups. The standard errors of the differences in the proportions show that all differences between the Negroes and the Panamanians, and between the Negroes and whites are valid, while the differences between the Panamanians and whites may be due to chance. Pyelonephritis is twice as common in the West Indians as it is in the other two races; this difference is especially noticeable in healed pyelonephritis which occurred three times as often in the West Indians as in the other groups. Arteriolonephrosclerosis is more than seven times as common in the Negroes as in the Panamanians and three

times as common in the Negroes as among the whites. The only other point to be noted is that almost 5 per cent of the total number of West Indians and whites had hypertension of slight degree without renal pathologic changes.

Correlation of Nephritis with Blood Pressure

Table IV shows that the highest blood pressures were recorded in Negroes with healed pyelonephritis and in whites and Negroes with nephrosclerosis. It is fairly consistently true in all groups that the West



Text-Fig. 1. Percentage incidence of nephritis in total autopsy group.

Indians had the highest blood pressures, the Panamanians had the lowest, and the whites fell between. The average blood pressures of the nephritic patients were: West Indians, 176/104; Panamanians, 136/89; and whites, 159/98. A correlation between the severity of nephritis and the blood pressure is evident in the following groups, which are the only ones large enough to analyze: Negro, pyelonephritis, severe, 187/110; and mild, 171/104; Negro, nephrosclerosis, severe, 189/110; and mild, 177/105.

The relative incidence of hypertension in the racial groups also may

be demonstrated by computing the percentage of patients in the total groups with diastolic blood pressures over 100 mm. of Hg, as shown in Table V. According to this criterion there were six times as many hypertensive patients among the Negroes as among the Panamanians, and three times as many among the Negroes as among the whites.

Causes of Death in Study Group

Table VI shows the causes of death in the hypertensive and nephritic cases classified according to the International List of the Causes of Death. It is apparent that most of the West Indians and whites died of conditions which were related to the cardiovascular or renal systems. Most of the Panamanians died of acute or chronic infections.

TABLE V
Incidence of Hypertension
(Cases with Diastolic Blood Pressure over 100 mm. of Mercury)

	Negroes	Panamanians	Whites
Number of cases	87	4	14
Percentage of total group	32.7	5.2	10.4
Percentage of nephritic group	66	33	50

REVIEW OF THE LITERATURE ON RACIAL INCIDENCE OF NEPHRITIS

General

The recognition of the co-existence of nephritis and hypertension is almost as old as the acceptance of these conditions as distinct clinical entities, but only recently has there been significant progress in understanding the causes for this relationship. The concept of renal hypertension which has grown out of much recent experimental and clinical evidence has stimulated many attempts to clarify the much argued pathologic classification of nephritis, particularly since the introduction of pyelonephritis as a cause of hypertension.

Lewis [5] has stated: "The literature on kidney diseases discriminates very little between those occurring in Negroes and in whites. The chief source of information is the various mortality statistics . . . Most of the mortality statistics fail to distinguish between the various forms of nephritis." In 1930 the mortality statistics for all the states showed one-sixth as much acute nephritis, about three-fourths as much chronic nephritis, and one-third as much unspecified nephritis in whites as in Negroes. Lewis noted that "it is clear that in each state the mortality of Negroes is much higher than that of whites." Statistics of the

Metropolitan Life Insurance Company,[17] 1911–1935, also showed a third as much acute nephritis and half as much chronic nephritis in whites as in Negroes, the rates for males and females being approximately equal.

In African Negroes, Hennessey[18] found that both cardiovascular diseases and nephritis were relatively uncommon, the most common

TABLE VI

Causes of Death in Study Group

	Negroes	Panamanians	Whites	Other races	Total
Hypertension and cardiac failure	26	1	4	1	32
Cerebral accident	19		5	2	26
Coronary accident	11		2		13
Pyelonephritis	12		1	1	14
Nephrosclerosis	1				1
Glomerulonephritis	1				1
Arteriosclerotic gangrene	3				3
Aneurysm	4				4
Malignant tumor	19		5		24
Cirrhosis	1		3		4
Pneumonia	7	1	1		9
Tuberculosis	6	2	1		9
Malaria		1	2		3
Leprosy	3	4			7
Syphilis	5	1			6
Typhoid and enteritis	2		1		3
Diabetes	2		1		3
Meningitis	2	1			3
Accidental	5		3		8
Blood dyscrasia			1		1
Thyrotoxicosis	1				1
Liver abscess	1				1
Surgical conditions of abdomen	12	1	4		17
Total	143	12	34	4	193

fatal nephritis being "proliferative hyalizing glomerulitis." Donnison[7] found no nephritis in 1800 native African hospital patients. In Yucatan and Guatemala, Shattuck[8,9] found a low incidence of nephritis among the native Indians. In Panama the only previous approach to this question was Marvin and Smith's[2] observation that of their hypertensive patients 94.1 per cent of the Panamanians and 83.3 per cent of the West Indians had abnormal urinary constituents. Of their patients, 84.4 per cent also showed diminished excretion of phenolsulfonephthalein.

Glomerulonephritis

In spite of the intensive study which glomerulonephritis has received, there is very little information available on its geographic and racial distribution. Seegal, Seegal, and Jost[19] found that in four latitude areas in the United States there was no significant variation in the incidence of

acute glomerulonephritis. This was in sharp contrast to the decreased case rate of scarlet fever and rheumatic fever in the southern states.

Pyelonephritis

Longcope[11,20] and Weiss and Parker[12,13] have demonstrated that hypertension may develop in cases of active chronic and healed pyelonephritis. Their case studies are so convincing that there is little doubt that a causal relationship exists. It is, however, difficult to find exact data on the percentage of cases of hypertension which are initiated by pyelonephritis and there are very few studies which have taken racial differences into consideration. Weiss and Parker[12] felt that 15 to 20 per cent of cases of malignant hypertension were due to pyelonephritis. Dunn[21] found that patients dying of hypertensive heart disease had pyelonephritis in the following percentages: white men, 13.8; Negro men, 22; white women, 9.8; and Negro women, 42.6. Kinney and Mallory[22] found healed pyelonephritis in 14 per cent of 1,000 consecutive post-mortem examinations at the Boston City Hospital.

Most studies[23-27] show that a larger percentage of patients with pyelonephritis have hypertension than is found in control groups or in groups with other types of renal disease. In some studies,[28,29] however, no correlation between hypertension and pyelonephritis was found. Many reports[24,25,30,31] of an improvement in the hypertensive syndrome following the ablation of a diseased kidney have now appeared. Even such sceptics as Smith, Goldring, and Chasis[32] accepted some of these case reports although they threw out most of them as being inconclusive.

Various estimates of the importance of chronic pyelonephritis as a cause of the contracted kidney of chronic Bright's disease have been made. Weiss and Parker[12] stated that 30 to 35 per cent of bilateral contracted kidneys were due to pyelonephritis. Ellis[33] found 13 examples of chronic pyelonephritis in 45 contracted kidneys. Staemmler[34] classified 55 contracted kidneys as follows: nephrosclerosis, 27; pyelonephritis, 18; and glomerulonephritis, 10.

Nephrosclerosis

Moritz and Oldt[35] found that 97 per cent of hypertensive patients had renal arteriolosclerosis and the remaining 3 per cent had severe renal arteriosclerosis. In contrast to this, only 12 per cent of nonhypertensive patients had arteriolosclerosis and the vascular lesions in all but 2 per cent of these cases were mild. A point of particular interest in relation to this study was their finding of 50 per cent more deaths due

to hypertension in Negroes than in whites. Other pathologic studies [27] have corroborated this correlation between renal arteriolosclerosis and hypertension. In a study of severely hypertensive patients whose kidneys were examined by biopsy in connection with operations for sympathectomy, Castleman and Smithwick [36] concluded that renal arteriolosclerosis was probably secondary to hypertension. Seven per cent of their patients showed no nephrosclerosis and in 53 per cent the vascular changes were so slight that they scarcely could have caused sufficient renal ischemia to produce hypertension. They did not study forms of nephritis other than nephrosclerosis.

Discussion

In a comparison between these published data and the findings in Panama several significant facts become evident. Nephritis in Panama differs from that in temperate zones, first, in the relative absence of glomerulonephritis. Almost all of the cases of glomerulonephritis in this series were mild and acute, and in only one case was it recorded as the cause of death. No cases had progressed to show sufficient evidence of chronic glomerulonephritis to permit that diagnosis histologically. This is probably associated with the fact that most manifestations of streptococcal infections are generally believed to be both rare and mild here. Tucker, Miller, and Kean [37] have found that the incidence of scarlet fever in Panama is lower than in the United States and that the diagnosis is made five times more frequently in whites than in Negroes.

Pyelonephritis is the most common form of nephritis in these patients. As would be expected in autopsy reports, most of the cases are of healed or active chronic pyelonephritis. This high incidence of pyelonephritis is most marked among the few Panamanians who had nephritis and almost all of these patients also had a concurrent chronic infection such as leprosy or tuberculosis. Thirty-two per cent of the nephritis in whites is due to healed or chronic pyelonephritis, which is about the same as the published estimates of the number of cases of chronic Bright's disease which are caused by pyelonephritis elsewhere. The incidence of pyelonephritis is even higher, 39 per cent, among the West Indians. This difference between whites and Negroes is not as marked as in Dunn's [21] report, and the distribution according to sex is the converse of that shown in her data. The racial difference is accentuated, however, when the percentages are derived using the total group of patients as a base rather than the study group. The percentage of Negroes with healed and active chronic pyelonephritis is

19.2 as compared with 6.7 per cent of the whites. The reason for this striking racial difference is not apparent. It is probably not a matter of difference in hygiene, cleanliness, sanitation, or prevalence of venereal disease because the incidence rate is approximately the same in Panamanians and whites, and if there is any difference between Negroes and Panamanians in any of these factors this difference probably favors the Negroes. The average blood pressures of the racial groups with pyelonephritis also show significant differences. The blood pressure of the Negroes is somewhat higher than that of the whites and considerably higher than that of the Panamanians. This suggests that other factors may be responsible for the development of hypertension in the Negroes and whites, or possibly that there is a varying intrinsic susceptibility in the races. However, this may be merely a reflection of the severity of the pathologic change. Among the Negroes, 48 per cent of the cases of pyelonephritis were classed as severe as compared with 36 per cent in the whites and 28 per cent in the Panamanians.

The most marked racial difference in the incidence of any form of nephritis is in the occurrence of nephrosclerosis in Negroes and Panamanians. The fact that this condition is over seven times as common in the Negroes as in the Panamanians is a significant corollary to the similar ratio in the incidence of hypertension in these groups. The whites again occupy an intermediate position. The differences in the blood pressure levels of the racial groups are about the same as with pyelonephritis. Again a similar difference in the severity of nephritis is observed.

Another point that merits consideration is the occurrence of hypertension in the absence of nephritis. Among both the Negroes and whites this is true in almost 5 per cent of the total number of cases autopsied or in 12 per cent and 18 per cent respectively of hypertensive patients. The hypertension in these cases was mild. This suggests that "essential hypertension" may occur in these two groups, while if a Panamanian develops hypertension it is probably on a renal basis.

Conclusions

Previous studies have shown that hypertension is five to eight times as frequent in West Indian Negroes living in Panama as in native Panamanians. From this analysis of 498 comparable autopsy cases the following conclusions seem to be justified.

1. Among the Negroes, hypertension (diastolic blood pressure over 100 mm. of Hg) was six times as common as in Panamanians and three times as common as in whites.

2. Among the Negroes there was three times as much nephritis as among the Panamanians and almost two and one-half times as much as among the whites.

3. The average blood pressures of the nephritic patients were: West Indians, 176 mm. of Hg systolic and 104 diastolic; Panamanians, 136 mm. systolic and 89 diastolic; and whites, 159 mm. systolic and 98 diastolic. Although this may mean that there is an actual difference in the susceptibility of the races to hypertension, it is also true that the severity of the nephritis in the three races showed a general correlation with the level of the blood pressure.

4. Glomerulonephritis comprised about 1 per cent of the nephritis in all racial groups and was never recognized in its chronic stages in this series.

5. Pyelonephritis was the most frequent form of nephritis in all groups and it was twice as common among the Negroes as among either of the other groups.

6. Healed pyelonephritis was three times as common among Negroes as among either of the other two groups.

7. Pyelonephritis comprised 58 per cent of the nephritis in Panamanians and most of these patients had concurrent leprosy or tuberculosis.

8. There was more than seven times as much arteriolonephrosclerosis among West Indians as among Panamanians and three times as much as among the whites.

9. No significant correlation between nephritis and sex distribution was observed.

10. Five per cent of the Negroes and whites had mild hypertension with no evidence of nephritis.

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EXPERIMENTAL STUDIES IN CALCIFICATION

IV. The Effect of Irradiated Ergosterol and of Starvation on the Dentin of the Rachitic Rat *

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McCollum, Simmonds, Shipley, and Park,[1] in 1922, discovered that starvation had the same healing effect on the rachitic epiphyseal cartilage as the administration of suitable amounts of cod-liver oil. It seemed, therefore, of interest to investigate the reaction of rachitic dentin to starvation and to compare the effect with that produced by vitamin D.

Review of the Literature

Weinmann,[2] in 1930, reported the changes in the dentin of rachitic rats following daily administration of 5 mg. of vigantol (vitamin D). The first effect was seen 1 day after administration in the incisal portion of the incisor where the pulpal layer of the dentin was deeply stained with hematoxylin. Under continued administration of vitamin D the hypercalcified layer extended toward the middle portion of the incisor. The basal predentin which was very long in rickets showed progressive calcification following the administration of vitamin D.

Irving[3] studied the dentin of rachitic rats 10 days after administration of 9.2 international units of vitamin D. He found that the dentin matrix laid down 4 to 6 days after administration was deeply stained with hematoxylin and that the predentin laid down prior to vitamin D treatment was either completely unchanged or else showed a few scattered calcified globules.

No references are available on the effect of starvation on either the normal dentition or that in rachitis.

Material and Methods

This report is based on the study of the upper jaws of four groups of white rats † which were sacrificed at 49 to 52 days of age. Group I: Four rats were fed a rachitogenic diet for 4 weeks (Steenbock and Black[4]) and were given 3 cc. of irradiated ergosterol 1 to 4 days before sacrifice (Table I). Group II: Four litter-mates of the animals of group I were fed a rachitogenic diet for 4 weeks and were starved for a period of 1 to 4 days (Table II). Group III: Three rats fed a

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† The animals used in this series of study were kindly made available by Drs. F. C. McLean and W. Bloom.

rachitogenic diet after weaning. Group IV: Three rats fed a basal diet. The control groups III and IV included litter-mates of groups I and II.

The heads of the experimental and control rats were removed and fixed in 10 per cent formalin immediately after sacrifice. The jaws were dissected and the upper incisors and molars were prepared for de-

TABLE I

Data on Four White Rats Placed on a Rachitogenic Diet and Then Given 3 cc. of Irradiated Ergosterol

Number	Age when sacrificed	Duration of rachitogenic diet	Time elapsed after last administration of ergosterol
	(days)	*(days)*	*(hours)*
2904	49	28	10
2905	50	29	22
2906	51	30	46
2907	52	31	70

calcified histologic sections. The incisors were cut in serial longitudinal sections and the molars in serial mesiodistal sections. The stains used were hematoxylin and eosin.

FINDINGS

Fasting or the administration of vitamin D resulted in an improvement in the calcification of the dentin. This effect was best observed in the basal predentin of the incisor. In this tooth a layer of predentin

TABLE II

Data on Four White Rats Kept on a Rachitogenic Diet and Subjected to Fasting

Number	Age when sacrificed	Duration of rachitogenic diet	Duration of fasting
	(days)	*(days)*	*(hours)*
2908	49	28	12½
2909	50	28	36½
2910	51	28	60
2911	52	28	84

is normally found along the entire pulpal surface as long as the latter is lined by active odontoblasts. The predentin layer is adjacent to the calcified dentin except at the most basal end where the predentin is next to the dentino-enamel or dentino-cemental junction. In this region the predentin just recently deposited has not yet calcified. This basal portion of the predentin layer is normally 0.1 to 0.2 mm. in length and will be referred to as the basal predentin (Fig. 1).

In rachitic animals the basal predentin is of considerable length, especially on the cementum-covered side of the tooth [5] (Fig. 2).

The earliest changes following the administration of vitamin D were seen at the end of the first day and, after fasting, on the second day. These consisted of an improvement in the calcification of the predentin, especially the basal predentin (vitamin D, Fig. 3; and starvation, Fig. 5). One or 2 days later the calcification of the outer layer of the basal predentin extended almost to the basal end of the incisor (Figs. 4 and 6).

Intermediate Dentin. A small zone of intermediate dentin is present in the middle third of the enamel-covered wall in the incisor of the normal rat but is missing in almost all rachitic rats. This layer of intermediate dentin reappeared beginning with the end of the first day of fasting and on the second day of vitamin therapy. It extended also to the incisal half of the basal third (Figs. 9 and 11). The predentin next to the intermediate dentin contained isolated globules (Figs. 9 and 10) which stained like the intermediate dentin (globular predentin, Schour and Rogoff [6]).

Incisal Dentin. In the incisal third of the incisor the dentin immediately adjacent to the predentin stained deeply with hematoxylin. This stripe increased in width and might extend into the middle third of the tooth with increase in the duration of the experiment.

Discussion

That fasting causes a reaction in the rachitic dentin identical to that induced by vitamin D is in complete agreement with the findings of McCollum *et al.*[1] on the changes in the epiphyseal cartilage. In both cases vitamin D or fasting improved the calcification of affected tissues. We can fully agree with their explanation of the effect of fasting on healing of rickets. They stated: "Just as soon as the load of a defective diet is removed and the body is forced to draw on its own tissues for maintenance of life and function, stored foodstuffs are released into the blood stream as the result of a process of selective tissue decomposition."

Cavins [7] noted that the fasting of rachitic rats induces a sharp rise in the level of inorganic phosphorus of the blood. In the light of our findings [8] and those of McLean and McCoy,[9] that following injections of phosphate solution the uncalcified hard tissues of rachitic rats started to calcify, it can be assumed that the liberation of phosphate compound from the tissues induces calcification in fasting rats.

The basal predentin is an excellent site for the study of the beginning of calcification of dentin. The earliest attempt at calcification starts in the oldest layers of the rachitic predentin.

The fact that the layer of intermediate dentin disappears in rickets

and reappears in our experiments which initiate a healing of the rachitic condition suggests that intermediate dentin can be considered a stage in the normal calcification of dentin. Its normal presence in the middle third of the convex wall of the incisor seems to show that in this region the conditions for the calcification of the predentin are normally more favorable than in the other parts of the tooth. In this zone the peripheral layer of the predentin has undergone partial calcification, while the predentin in other parts of the tooth is uncalcified in its entire width. The calcification in this area is more rapid and the predentin, therefore, is narrower. In fact, the width of predentin plus intermediate dentin is equal to the width of the predentin in the basal and incisal areas.

The preferred location of the intermediate dentin in the middle third of the tooth in normal rats may be explained on the basis of the vascular supply of this region. It is known that calcification is retarded in richly vascularized areas and in areas lying in close proximity to blood vessels. The vascularization of the middle third of the pulp seems to be optimal for calcification because it is not as rich as that of the basal third, whereas the blood vessels in the incisal third of the pulp show regressive changes.

The fact that the calcification of rachitic dentin improves during fasting may explain in part the inconsistency in calcification observed in the dentin of various experimental animals. The possible effects of spontaneous fasting of animals during short-term experiments must be kept in mind.

Our experiment was restricted to a period of 4 days following the first administration of vitamin D. The observation that an attempt at calcification in this period starts in the oldest layers of rachitic predentin does not contradict the findings of Irving [3] who investigated the incisor 10 days after administration of vitamin D. He wondered why "the old predentin was not more altered, seeing that this was being calcified, though with difficulty, while the animal was on the deficient diet alone." However, his photomicrographs of fields taken from the middle and labial portion of the incisor show a layer of intermediate dentin adjacent to the peripheral layer, which calcified during the rachitic regime.

Summary

The dentin of incisors of four albino rats placed on a rachitogenic diet and given 3 cc. of irradiated ergosterol, and of four litter-mates kept on a rachitogenic diet and subjected to fasting for different periods was studied histologically. The histologic findings were:

1. Fasting and the administration of vitamin D cause identical reparative changes in the dentin of rachitic rats.

2. The first calcification of the wide rachitic predentin begins in its oldest layer and is best seen in the basal predentin.

3. The layer of intermediate dentin which has disappeared during rickets redevelops in the basal third of the enamel-covered dentin.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 184.

Photomicrographs of longitudinal sections of the basal end of the lingual side of upper incisors. The area is indicated in the insert in Figure 1. B.pd. = basal predentin; Od. = odontoblasts; Pdm. or Per. = periodontal membrane; Al.B. = alveolar bone; P = pulp. × 48.

FIG. 1. Rat 2901, 49 days of age, placed on normal basal diet. The predentin is thin and the basal predentin is of very short extent (0.1 mm.).

FIG. 2. Rat 2903, 49 days of age, placed on rachitogenic diet for 28 days. Here are seen the thick layer of predentin; the long basal predentin (1.5 mm.); and the narrowing of the width of the dentinal wall at the area where calcification of the dentin starts.

FIG. 3. Rat 2905, 50 days of age, placed on rachitogenic diet for 29 days, and given 3 cc. of irradiated ergosterol 22 hours before sacrifice. A thin stripe of calcified dentin extends halfway into the basal portion, which is otherwise formed by basal predentin only (0.8 mm.).

FIG. 4. Rat 2906, 51 days of age, placed on rachitogenic diet for 30 days and administered 3 cc. of irradiated ergosterol 46 hours before sacrifice. The increased length of the calcified stripe in the otherwise uncalcified basal portion (0.6 mm.) may be compared with that in Figure 3.

FIG. 5. Rat 2909, 50 days of age, placed on rachitogenic diet for 28 days and subjected to fasting for 36½ hours. There is narrowing of the dentinal wall and a thin stripe of calcified dentin in the basal portion. The basal predentin is 0.7 mm. long.

FIG. 6. Rat 2911, 52 days of age, placed on rachitogenic diet for 28 days and subjected to fasting for 84 hours before sacrifice. The increased calcification in the basal portion may be compared with that in Figure 5. The basal predentin is 0.4 mm. long.

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Effects of Ergosterol and Starvation

PLATE 185

Photomicrographs of longitudinal sections from the basal third of upper incisors. The area is indicated in the insert in Figure 7. D = dentin; Pd. = predentin; Od. = odontoblasts; I.D. = intermediate dentin. × 610.

FIG. 7. Rat 2901, 49 days of age, kept on basic diet. Of note are the wide, fairly evenly calcified portion of the dentin and the narrow stripe of predentin.

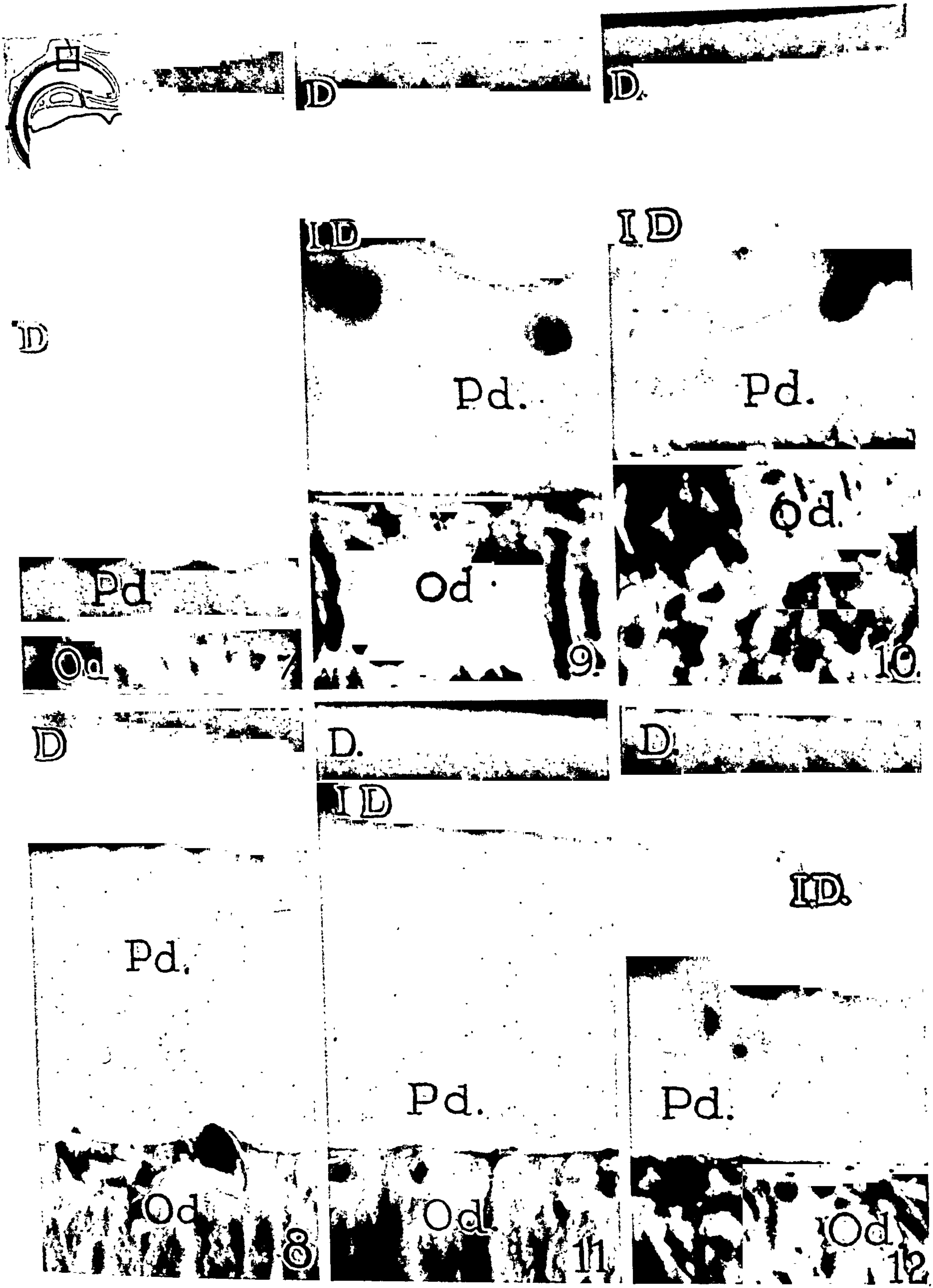
FIG. 8. Rat 2903, 49 days of age, placed on rachitogenic diet for 28 days. The extensive width of the predentin, the narrow portion of the calcified dentin, and the reduced width of the total dentin wall may be compared with the normal appearance of Figure 7.

FIG. 9. Rat 2905, 50 days of age, placed on rachitogenic diet for 29 days and given 3 cc. of irradiated ergosterol 22 hours before sacrifice. A layer of intermediate dentin adjacent to the peripheral calcified dentin and globuli in the predentin are shown.

FIG. 10. Rat 2907, 52 days of age, placed on rachitogenic diet for 31 days and given 3 cc. of irradiated ergosterol 70 hours before sacrifice. The layer of intermediate dentin shows an increase in thickness and globuli are present in the predentin.

FIG. 11. Rat 2909, 50 days of age, placed on rachitogenic diet for 28 days and subjected to fasting 36½ hours before sacrifice. There is a layer of intermediate dentin adjacent to the pre-experimental calcified layer of dentin.

FIG. 12. Rat 2911, 52 days of age, placed on rachitogenic diet for 28 days and subjected to fasting 84 hours before sacrifice. To be noted are the increase in thickness of the layer of intermediate dentin and the reduced thickness of the predentin.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EXPERIMENTAL STUDIES IN CALCIFICATION

V. The Effect of Phosphate on the Alveolar Bone and the Dental Tissues of the Rachitic Rat *

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Elsewhere [1,2] we have described in detail the rachitic changes in the dentin of the rat incisor and have made an attempt to interpret the development of the rachitic deformities of the alveolar bone by studying the pathologic changes in those areas in which the normal growth pattern had been carefully analyzed. The purpose of this study was to trace the beginning of healing in selected areas of the rachitic alveolar bone and in the rachitic dentin following injections of phosphate solutions.

Review of Literature

Lilly, Peirce, and Grant [3] reviewed the literature, up to 1935, on the effect of phosphorus on bones. They observed that healing of rickets by feeding phosphates is comparable to that produced by vitamin D.

McLean and McCoy [4] injected sublethal doses of phosphate (7.5 mg. of phosphorus per 100 gm. of body weight) in the form of a 1/10 molar mixture of NaH_2PO_4 and Na_2HPO_4 at pH 7.35 into 49-day-old rachitic rats. Histologically they observed beginning calcification in the cartilage matrix in the majority of the animals killed at the end of 4 hours, and almost uniformly after 8 hours. The deposit of calcium increased in density up to 24 hours. Urist and McLean [5] reported that calcification was initiated in the epiphyseal cartilage within 24 hours after the injection of phosphate solution. Within 48 hours, and following two injections, calcification was usually observed in the osteoid zone in the metaphysis, and in the osteoid borders on the old trabeculae of spongy bone at the junction of the rachitic metaphysis with the shaft. They observed further that shortly after calcification began in the cartilage, orderly penetration and removal of cartilage by advancing capillaries also began.

The effects of phosphates on the dental and alveolar structures have not been reported so far as we are able to ascertain.

Materials and Methods

This study is based on 12 rats † which were placed on a rachitic diet (Steenbock and Black [6]) at the time of weaning (21 days) and given,

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† The animals used in this series of studies were kindly made available by Drs. F. C. McLean and W. Bloom.

beginning at the age of 49 days, single or multiple daily intraperitoneal injections of phosphate solutions. The animals were sacrificed from 6 hours to 6 days following the first injection (Table I).

The standard phosphate solution was a mixture of 80 per cent of 1/10 molar secondary sodium phosphate (M/10 Na_2HPO_4) and 20 per cent of 1/10 molar primary sodium phosphate (M/10 NaH_2PO_4) of which the hydrogen-ion concentration at 38° C. corresponded approximately to pH 7.35. The standard dosage employed was 2.5 cc. (containing approximately 7.5 mg. of phosphorus) per 100 gm. of rat weight. This dose is subtoxic for rachitic rats, rarely resulting in the death of an animal in tetany (McLean and McCoy[4]).

TABLE I

Data on Twelve Albino Rats Placed on a Rachitogenic Diet after Weaning and Treated with Phosphate 1 to 6 Days before Sacrifice

Rat number	No. of injections	Doses of 1/10 molar phosphate solution	Age at death	Duration of experiment
		(cc.)	(days)	
405	1	1.30	50	1 day
406	2	1.35, 0.67	51	2 days
407	3	1.27, 0.67, 1.27	52	3 days
408	4	1.37, 0.68, 1.37, 1.45	53	4 days
409	5	1.32, 0.66, 1.32, 1.30, 1.30	54	5 days
412	6	1.87, 0.93, 1.87, 2.02, 2.02, 2.02	55	6 days
3805	1	1.05	49	6 hours
3804	1	1.05	50	1 day
3806	2	1.10, 0.85	51	2 days
3807	3	1.15, 1.15, 1.15	52	3 days
3808	4	1.02, 1.02, 1.02, 1.02	53	7 days
3809	5	2.50, 2.50, 2.50, 2.50, 2.50	54	5 days

The heads of the experimental and control rats were removed and fixed in 10 per cent formalin immediately after sacrifice. The jaws were dissected and the upper incisors and molars were prepared for decalcified histologic sections. The incisors were cut in serial longitudinal sections and the molars in serial mesiodistal sections. The stains used were hematoxylin and eosin.

FINDINGS

Alveolar Bone

Lingual Alveolar Plate of the Upper Incisor. In the upper incisor of the normal rat the middle portion of the lingual alveolar bone consists of a plate approximately 0.1 mm. thick, which separates the periodontal membrane from the maxillary sinus (Fig. 1). The periodontal surface of this plate is covered by a continuous row of osteoblasts. Its nasal surface is covered by numerous osteoclasts and is continuously

resorbed, apparently in conjunction with the growth of the maxillary sinus. The alveolar plate is perforated by a few vessels.

In advanced cases of rickets this plate has increased to about double its normal size and consists completely of osteoid tissue. A layer of osteoblasts on its periodontal surface indicates progressing apposition. The nasal surface is covered by spindle-shaped connective tissue cells and is devoid of osteoclasts and Howship's lacunae. This suggests that resorption on this surface has come to a standstill.

No significant changes were observed in this area 24 hours after a single injection of phosphate solution (Fig. 3). Following two daily injections of phosphate, islands of calcification could be observed on the nasal surface (Fig. 2). Adjacent to the calcified areas, the connective tissue cells differentiated into osteoclasts lying in shallow lacunae. Three days later the calcification had almost reached the periodontal surface. Seams of osteoid tissue remained adjacent to the penetrating vessels and to the periodontal surface (Fig. 4).

Fundic Bone of the Incisor. Normally the alveolar bone at the fundus is formed by a cup-shaped lamella approximately 0.05 mm. in thickness (Fig. 5). The bone is continuously resorbed on the surface facing the incisor. At the nasal surface, which is bordered by the mucous glands of the nasal cavity, apposition is in progress. In cases of severe rickets the lamella is twice as thick as is normal and consists entirely of osteoid tissue. The adjacent connective tissue of the periodontal membrane has degenerated and is transformed into hyaline tissue, forming strands and irregular trabeculae. Groups of connective tissue cells are included between the hyaline trabeculae. The border between osteoid and hyaline tissue is indistinct. The hyaline connective tissue is eosinophilic, as is the osteoid tissue.

Six hours after the injection of phosphate the tips of the hyaline trabeculae at the apical periodontal tissue stained with hematoxylin, indicating the beginning of calcification; 18 hours later the area of calcification had markedly widened (Fig. 6). One day later osteoclasts appeared at the periodontal surfaces and between the hyaline trabeculae (Fig. 7). The osteoclasts increased in number during the subsequent days of the experiment (Fig. 8). The hyaline trabeculae were reduced to small islands. They were surrounded by connective tissue which had replaced the hyaline tissue. The osteoclasts were found adjacent to the remnants of hyaline tissue or in the connective tissue. On the fifth day (Fig. 9) the hyaline tissue had almost entirely disappeared. Large osteoclasts had approached the previous border between hyaline and osteoid tissue which appeared to be slightly calcified in its oldest layer. On the sixth day the entire bony lamella had calcified ex-

cept for a thin osteoid seam at the nasal surface. The osteoclasts were attacking the plate proper (Fig. 10).

A similar reaction to the injection of phosphate could be observed in the premaxillary part of the alveolar bone at the convex side. In this region a hyalinization of the alveolar periosteum and compression of the enamel organ are found in severe cases of rickets.[2] Following injections of phosphate, the hyaline tissue calcified, was removed by osteoclasts, and the compressed enamel organ recovered.

Alveolar Bone of the Molars. In cases of severe rickets the lamina dura around the molars is formed by a layer of osteoid tissue. It is thickest at the crest of the septa. The continued apposition of osteoid tissue at the crest of the interradicular septa leads to an obliteration of the periodontal membrane when the vertical eruption of the molars ceases.[2]

Between the third and fifth days after the administration of phosphate the osteoid tissue began to calcify in its deepest layer where it bordered on the pre-experimental calcified core of the septa. The newly calcified bone was not as darkly stained with hematoxylin as was the pre-experimental bone. In the areas close to the periodontal surface the calcification was not even, because the osteoid tissue, in which bundles of Sharpey's fibers are contained, calcifies later than the areas between the bundles.

The obliteration of the periodontal membrane at the bifurcation of the molars began to disappear in some animals on the fourth day of phosphate administration. The width of the osteoid tissue covering the trabeculae of the spongy bone in the alveolar process gradually diminished during the period of phosphate administration.

Dentin of the Upper Incisor

The rachitogenic diet causes not only a retardation in calcification of the dentin but also a marked decrease in dentin formation. This is indicated by the widening of the predentin layer and a decrease in the total width of dentinal wall.[1]

Following phosphate injection, not only was the calcification of the predentin speeded up but also the formation of dentin was considerably accelerated (Table II). The intermediate dentin which disappears while on the rachitogenic diet reappeared after phosphate administration, just as was observed after administration of vitamin D or after fasting.[7] It was found not only in the middle third of the enamel-covered dentin, as in normal animals, but extended into the incisal part of the basal third. The basal predentin of the incisor which is entirely

uncalcified in rachitic animals calcified during the first 3 days of phosphate administration.

Discussion

McLean and McCoy[4] established that the epiphyseal cartilage of rachitic rats begins to calcify as early as 4 hours after injection of phosphate. Calcification of the osteoid tissue at the alveolar process could not be seen in our material until the end of the second day following phosphate administration. The first layers to calcify were the oldest layers of the osteoid tissue. Resorption of the calcified osteoid tissue by osteoclastic activity occurred at a slow rate.

The hyaline tissue which was formed by the degeneration of the compressed connective tissue differed markedly in its behavior from that of osteoid tissue, although microscopically these two tissues appeared very similar. The hyaline tissue showed signs of calcification, almost simultaneously with the epiphyseal cartilage. The layers of hyaline tissue which had been formed *last* were the first to calcify. After calcification the hyalin was speedily removed by osteoclastic resorption. These observations show biologic differences in the nature of the osteoid and the hyaline tissues.[2]

Table II

*Width * in Microns of Predentin, Calcified Dentin, and Dentinal Labial Wall of Rachitic Rats Given Intraperitoneal Injections of Phosphate*

Rat no.	Duration of experiment	Predentin	Calcified dentin	Labial dentinal wall
	(*days*)	(μ)	(μ)	(μ)
3805	1	61.6	31.3	92.9
3804	2	49.7	68.1	117.8
3806	3	47.8	75.4	123.2
3807	4	41.4	83.7	125.1
3808	5	34.0	100.3	134.3
3809	6	34.0	118.7	152.7

* Measured at the level of completed formation of enamel matrix and representing an average of three measurements.

The disappearance of the compression of the periodontal membrane between interradicular septa and molars was in all probability due to the resumption of the endochondral growth in the mandibular condyle. The distance between maxillary and mandibular bodies was again increased and space was opened for the vertical eruption of the molars.

The action of phosphates on the dentin was identical with that of vitamin D and fasting,[7] both of which induce a rise in the level of the inorganic phosphorus of the blood.

The findings in this study and by previous investigations show that

intraperitoneal injections of phosphate lead to a healing of rickets in the absence of vitamin D. These evidences support the assumption that one rôle of vitamin D is the increase of the phosphorus level of the blood.

SUMMARY

The effect of intraperitoneal injections of phosphate on the alveolar bone and the dentin was studied histologically in 12 rachitic rats. The findings were:

1. Osteoid tissue begins to calcify at the end of the second day after the administration of phosphate; the calcification begins in the oldest layers of the osteoid tissue.
2. The hyaline tissue formed by the degeneration of connective tissue shows the first signs of calcification almost immediately after injection of phosphate; the first layers to calcify are those which have been formed last.
3. After calcification of osteoid and hyaline tissues, osteoclasts reappear and resorb the calcified tissues; the resorption of calcified hyaline tissue progresses at a faster rate than that of calcified osteoid tissue.
4. Beginning with the fifth day of the experiment the encroachment of the periodontal membrane between molars and interradicular septa disappeared. This is probably caused by the resumption of the vertical eruption of the molar correlated with the resumption of condylar mandibular growth.
5. The dentin shows a resumption of normal calcification and a normal rate of formation.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 186

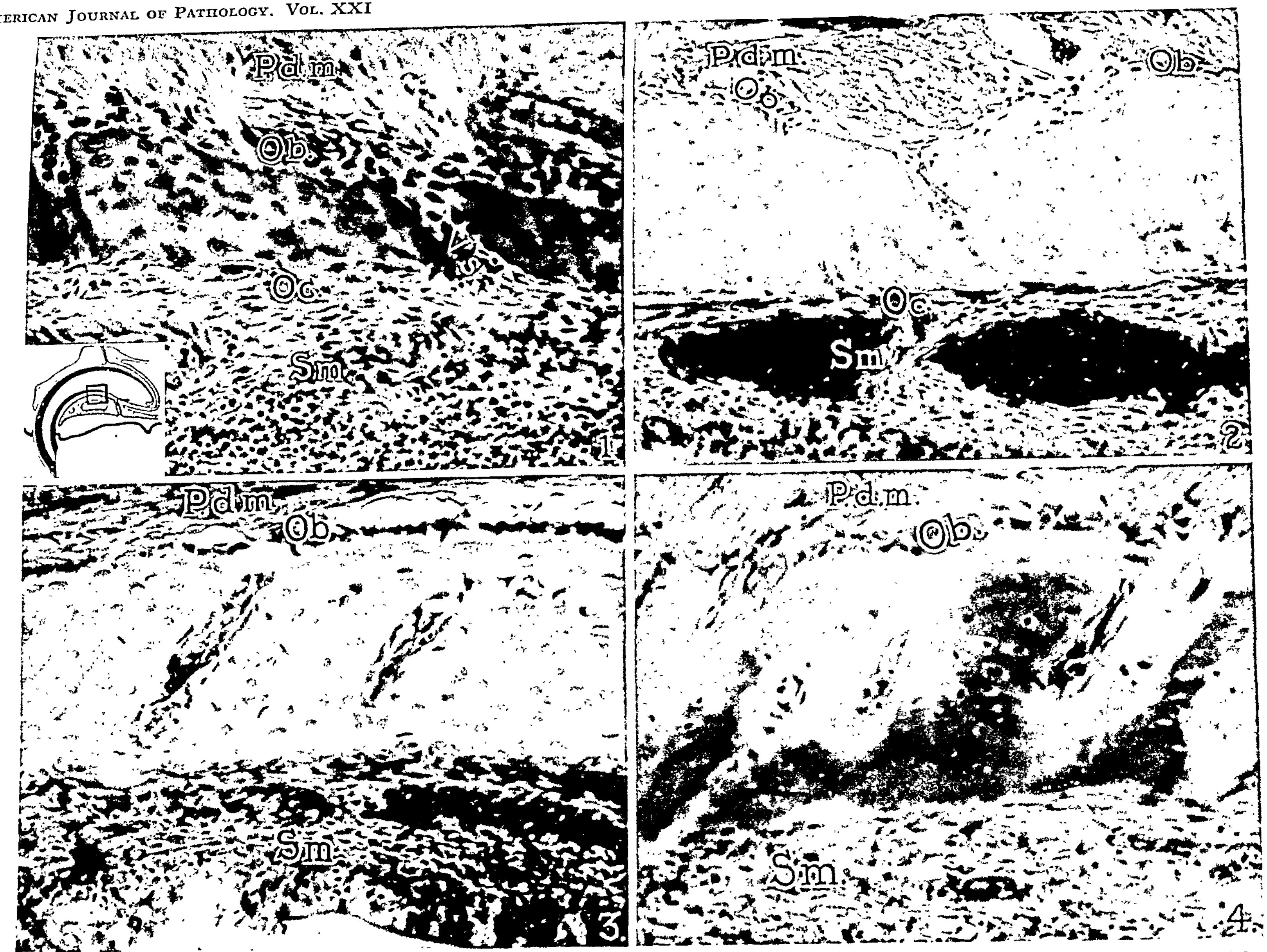
Photomicrographs of the lingual alveolar bone. The area is indicated in the insert in Figure 1. Ob. = osteoblasts; Oc. = osteoclasts; P.d.m. = periodontal membrane; Sm. = submucosa; V.s. = vessels penetrating the bony lamella. × 214.

FIG. 1. Rat 3801, 54 days of age, placed on normal basic diet. The normally calcified bony lamella is seen. Osteoblasts facing the incisor indicate progressing apposition; osteoclasts on the side of the nasal cavity indicate resorption.

FIG. 2. Rat 3806, 51 days of age, placed on rachitogenic diet for 30 days and sacrificed 2 days after two intraperitoneal injections of phosphate. At the nasal surface of the bony plate beginning calcification and resorption are seen.

FIG. 3. Rat 3804, 50 days of age, placed on rachitogenic diet for 29 days and sacrificed 24 hours after a single intraperitoneal injection of phosphate. The bony lamella is about twice as thick as normal, consisting entirely of osteoid tissue. Osteoblasts indicate progressive apposition.

FIG. 4. Rat 3809, 54 days of age, placed on rachitogenic diet for 33 days and sacrificed 5 days after five intraperitoneal injections of phosphate. Calcification is in an advanced stage. The osteoid layer persists next to the periodontal surface and penetrating vessels.



Weinmann and Schour

Effect of Phosphate

PLATE 187

Photomicrographs of the fundic bone. The area is indicated in the insert in Figure 5. P.d.m. = periodontal membrane; Mg = mucous glands of the nasal cavity. × 233.

FIG. 5. Rat 3801 placed on normal basal diet. The normally calcified bony lamella shows bone apposition at the nasal surface and resorption at the periodontal surface.

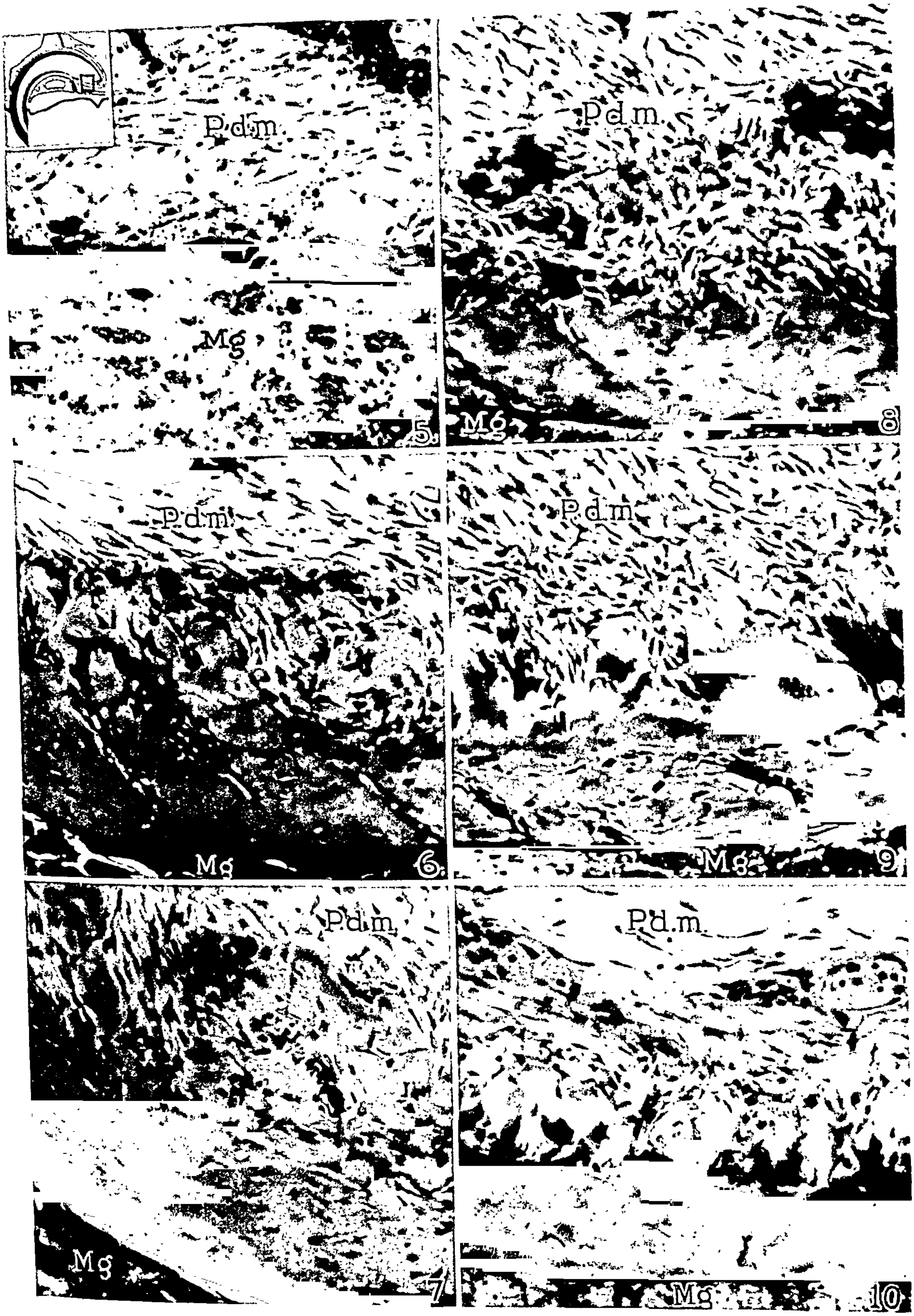
FIG. 6. Rat 405, 50 days of age, placed on rachitogenic diet for 29 days and sacrificed 24 hours after a single intraperitoneal injection of phosphate. The bony lamella is about twice as thick as normal, consisting entirely of osteoid tissue, and a large portion of the periodontal membrane is degenerated and transformed into hyalin. The tips of hyaline trabeculae are stained with hematoxylin, indicating beginning of calcification.

FIG. 7. Rat 406, 51 days of age, placed on rachitogenic diet for 30 days and sacrificed after two intraperitoneal injections of phosphate. Osteoclasts appear between and at the periodontal surfaces of hyaline trabeculae.

FIG. 8. Rat 408, 53 days of age, placed on rachitogenic diet for 32 days and sacrificed after four intraperitoneal injections of phosphate. The fibrous tissue contains numerous osteoclasts which have resorbed hyaline trabeculae, leaving only small strands of hyaline tissue.

FIG. 9. Rat 409, 54 days of age, placed on rachitogenic diet for 33 days and sacrificed after five intraperitoneal injections of phosphate. Fibrous tissue has replaced hyaline trabeculae almost completely and osteoclasts are bordering the fundic bone at the periodontal side.

FIG. 10. Rat 412, 55 days of age, placed on rachitogenic diet for 34 days and sacrificed after six intraperitoneal injections of phosphate. Osteoclasts are numerous at the periodontal surface of the fundic bone, which is stained with hematoxylin, indicating calcification, except for an osteoid layer at the nasal surface.


P.d.m.
Mg
5.
P.d.m.
Mg
8
P.d.m.
Mg
6.
P.d.m.
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9
P.d.m.
Mg
7
P.d.m.
Mg
10

INTESTINAL LIPODYSTROPHY (WHIPPLE'S DISEASE) *

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In 1907 Whipple [1] described for the first time a disease entity for which he suggested the term "intestinal lipodystrophy." His report was based on the case of a 37-year-old physician who had a 5-year history of progressive asthenia and weight loss, developed diarrhea and fatty stools a few months before death, and showed a striking lipoid deposition in the mesentery and small intestine. Since that time 6 additional cases have been reported which show the characteristic lesions described by Whipple. The present case is the eighth case to be reported.

Also of interest in this case are the bizarre hematologic findings that led to a diagnosis of atypical lymphatic leukemia, treated by radioactive phosphorus; and a subsequent diagnosis of hemolytic icterus, treated by splenectomy.

After Whipple's description in 1907, the next report of similar cases was that of Blumgart [2] (1923) who described 3 cases of progressive emaciation associated with steatorrhea. Jarco [3] (1936) reported a case similar to Whipple's which he stated was the third such case to be reported, since Jarco and others have accepted only one of Blumgart's cases (case 2) as belonging to this disease entity.

Reinhart and Wilson [4] (1939) and Sailer and McGann [5] (1942) have described cases which presented a histologic picture similar to that described by Whipple.[1] Sailer and McGann, in their review of the subject, included 4 cases (Fleischmann,[6] Bargen, Bollman, and Kepler,[7] Korsch,[8] Hill [9]) which they believed showed the characteristic syndrome. Sailer and McGann termed the disease lipophagic granulomatosis. Of the cases listed, only one (Korsch) seems to have had the characteristic changes of mesenteric nodes and bowel.

The most recent case is that of Pearse.[10] His patient related a 2-year history of recurring bouts of abdominal distention, intermittent crampy pain, and diffuse tenderness of the abdomen. At operation cysts filled with lipid were found in the peritoneum, omentum, intestines, and liver. Whipple personally examined the histologic sections of these lesions and stated that the lesions were similar to those he had originally described. Pearse's report does not mention mesenteric lymph nodes and no microscopic examination of the small bowel was made. This was the first diagnosis made ante-mortem and the first case to show changes in locations other than the abdominal nodes or small bowel.

* Received for publication, January 22, 1945.

Report of Case

The patient (B. C. H. no. 1,080,279), an Armenian male, 48 years old, entered the hospital on September 10, 1942, because of persistent diarrhea for 1 month. He had been followed in various Boston hospitals since June, 1941, when he entered a local hospital because of weakness, night sweats, and cough. At that time the spleen was felt 6 cm. below the left costal margin and extended to the mid-abdominal line. The hemoglobin was 11 gm. (Sahli); the red blood cell count, 4,630,000; hematocrit, 37; white blood cell count, 41,000, of which 80 per cent were large lymphocytes considered abnormal and unclassified. Oxidase stain 6 days later showed 61 per cent of the leukocytes to be oxidase-negative. The erythrocyte fragility test was considered to be within normal limits. The bleeding time was 1 minute; the clotting time, 10 minutes. The nonprotein nitrogen was 33; the total protein, 6.6; albumin, 4.5; globulin, 2.1 mg. per 100 cc. The basal metabolic rate was +24. Microscopic examination of a lymph node secured for biopsy showed well differentiated, moderately active germinal centers which were not anaplastic. There was no evidence of leukemia or neoplasm in this node. A diagnosis of hyperplasia was made. A specimen of the sternal marrow was interpreted as "hyperplasia of bone marrow." The clinical diagnosis was lymphatic leukemia with atypical cells. The patient was treated with radioactive phosphorus.

He was seen at this same hospital 7 months later because of persistence of weakness and cough. At that time he was slightly icteric; the spleen was down to the left iliac crest and extended across the midline. The liver for the first time was percussed 2 fingersbreadth below the right costal margin. The hemoglobin was 5.4 gm.; red blood cell count, 1,380,000; hematocrit, 18; platelets, 140,000 per cmm. The white blood cell count was 19,600 and 70 per cent of the white cells were lymphocytes, which were thought to be similar to those seen when the blood was first studied. A specimen of the sternal marrow again was interpreted as showing "hyperplasia of the marrow." The icteric index was 21. The patient was given multiple transfusions and was instructed to take iron and brewer's yeast.

On April 6, 1942, because of weakness, palpitation, dizziness, dyspnea, and occasional mild anterior chest pain, he was admitted to the II Medical (Harvard) Service of the Boston City Hospital. At the time of admission the temperature was 99° F.; pulse, 100; respirations, 24; blood pressure, 110/60 mg. Hg. He was a normally developed but poorly nourished, middle-aged Armenian who appeared to be chronically ill. There was moderate exophthalmos. Examination of the fundi showed multiple small, hemorrhagic areas, most marked about the vessels. The neck veins were distended. There were scattered moist râles at the lung bases. The heart was enlarged to the left, being in the anterior axillary line in the sixth interspace. There was a blowing systolic murmur over the entire precordium, but most marked at the apex. P_2 was louder than A_2. The liver edge was smooth and was felt well below the level of the umbilicus. The spleen was enlarged, smooth, and firm, and was also felt below the level of the umbilicus.

At the time of entry the hemoglobin * was 21 per cent (Sahli); red blood cell count, 750,000; white blood cell count, 34,000. The differential blood count gave polymorphonuclear leukocytes, 21 per cent; band forms, 5 per cent; eosinophils, 1 per cent; small lymphocytes, 15 per cent; large lymphocytes, 45 per cent; atypical lymphocytes, 9 per cent; monocytes, 2.5 per cent; metamyelocytes, 1 per cent; myelocytes, 0.5 per cent. There were 25 to 36 per cent reticulocytes, and the red blood cells showed variation in size with spherocytes and many large polychromatophilic cells. The hematocrit reading was 10; icteric index, 50 to 75; van den Bergh reaction (direct), 0.9 mg. per cent, (indirect) 7.6; D/I, 12 per cent. The

* The authors wish to thank Miss Geneva Darland for the hematological studies in this case.

blood platelet count was 73,000. Prothrombin was 100 per cent. The bleeding time was 3.5 minutes. The blood coagulation time was 5.5 minutes in glass and 11.5 minutes in lusteroid. The nonprotein nitrogen was 24 mg. per 100 cc. The total proteins were 5.12 mg. per 100 cc. The results of urine and stool examinations were negative.

Erythrocytic fragility as determined by resistance to hypotonic saline solution showed, on three successive examinations over a period of 3 weeks: 1 per cent hemolysis of the red cells in dilutions of 0.84 to 0.77; 10 per cent hemolysis at 0.67 to 0.61; 50 per cent hemolysis at 0.50 to 0.45; and 75 per cent hemolysis from 0.44 to 0.39. A normal control showed 1 per cent hemolysis at 0.45; 10 per cent at 0.41; 50 per cent at 0.38; and 75 per cent at 0.36 dilution. The acid hemolysis test, Donath-Landsteiner test, test for hemolysins, and test for cold agglutinins were all negative.

Three brothers, two sons, and a daughter of the patient showed normal fragility curves. It was believed that the patient was suffering from hemolytic jaundice and on May 1, 1942, a splenectomy was performed.

The spleen weighed 635 gm. and measured 26.3 by 16.3 by 5 cm. There were a few scattered fibrous adhesions over the capsular surface. Five slightly raised, irregular, yellow areas of infarction were present and these varied in size from 1 to 5 cm. in their greatest diameters. The remaining surface was smooth and dull red-purple-gray. There was a deep notch at each pole of the spleen. The pedicle measured 2 cm. in width. The splenic artery and vein were patent. The organ was firm. The cut surfaces were purple-gray and were discolored by the areas of infarction. The large blood vessels were prominent as were the trabeculations. The follicles could not be identified on the homogeneous purple surface.

Microscopic examination of sections of the spleen showed areas of infarction with adjacent areas of hemorrhage. Throughout the spleen there was intense engorgement of the pulp with compression of the follicles. Large spaces were lined with hypertrophied endothelial cells. There were deposits of hemosiderin scattered throughout the pulp. The follicles showed the usual structure.

Seven days after operation, the patient's erythrocytic fragility had decreased to 1 per cent hemolysis at 0.54 dilution, 75 per cent at 0.35. However, 1 month following operation 1 per cent hemolysis occurred at 0.70, and 75 per cent at 0.46, and the fragility remained near this level during the remainder of the hospital stay.

The week following operation the patient began to suffer from soreness and colicky pain in the abdomen and from anorexia without nausea or vomiting. The temperature was 100° F.; pulse, 88; respirations, 20. These symptoms continued and in addition the patient developed signs of infarction of the lower lobe of the right lung. A roentgenogram of the chest was consistent with this diagnosis. A gastrointestinal series was negative. A Graham series showing the gallbladder to be poorly filled by dye was considered to be evidence of disease of that organ. During the eleventh hospital week the patient's temperature reached 103° F. on two occasions and fell by lysis to normal in 7 days. At this time there was much abdominal distention with a slight increase in icterus, and a palpable, tender mass measuring approximately 5 cm. was found in the left upper quadrant. After 13 days of sulfadiazine administration the temperature fell to normal and remained

so thereafter. He was considered clinically to have had a subdiaphragmatic abscess.

It should be noted that 2½ months following operation the hemoglobin was 75 per cent; the red blood cell count, 3,360,000; the hematocrit, 33.8; and the white blood cell count 22,400, with the following differential count: polymorphonuclear leukocytes, 40.5 per cent; band forms, 6 per cent; basophils, 2 per cent; small lymphocytes, 8 per cent; monocytes, 7 per cent; and metamyelocytes, 0.5 per cent.

The patient was discharged on August 5, 1942, apparently well, to be followed in the out-patient clinic, but was readmitted to the hospital on November 22, 1942, because of diarrhea of 1 month's duration. Bowel movements varied from one to seven per day, the stools being green to brown and occasionally containing mucus but no blood. The patient complained also of epigastric pain, increasing dyspnea, weakness, and swelling of the ankles for the same period of time. The temperature was then 98.6° F.; pulse, 102; respirations, 22; blood pressure, 95/50. The heart was moderately enlarged to the left. There were dullness and decreased breath sounds over the bases of both lungs. The liver edge was tender and was felt 4 fingersbreath below the costal margin in the right midclavicular line. There was pitting edema of the lower extremities.

The patient was treated symptomatically. In addition, he was given enteric-coated sulfadiazine tablets, and a course of mercupurin with resulting diuresis and disappearance of ankle edema. The diarrhea persisted, with only occasional scattered days of freedom. After 2 months of unsuccessful hospitalization he became restless and left the hospital against the advice of the physicians.

Twenty-four hours after leaving the hospital the patient died suddenly, unattended by a physician.

During the final hospital admission the following laboratory data were secured: hemoglobin, 81 per cent (Sahli); red blood cell count, 3,800,000 per cmm.; hematocrit, 38.6 per cent; mean corpuscular volume, 101.6 cu. μ; mean corpuscular hemoglobin concentration, 32.7 per cent; mean corpuscular hemoglobin, 33.3 micromicrograms; reticulocytes, 2.9 per cent; icteric index, 5; white blood cells, 13,500 per cmm.; differential count: polymorphonuclear neutrophils, 45.5 per cent; polymorphonuclear neutrophils (band forms), 27.0 per cent; eosinophils, 1 per cent; basophils, 0.5 per cent; small lymphocytes, 9.5 per cent; large lymphocytes, 4 per cent; adult monocytes, 7 per cent; young monocytes, 3 per cent.

AUTOPSY FINDINGS

The body (M.I.P. no. A42-709) was that of a normally developed, emaciated, white male. The skin was sallow with slightly increased pigmentation consistent with the patient's Armenian lineage, but no icterus could be demonstrated. The body cavities were not remarkable. The heart and lungs were negative. The spleen was absent but a splenculus, 2 cm. in diameter, was found which presented the usual splenic structure. The liver was markedly enlarged and extended across the upper abdomen to the left midclavicular line. It extended 10 cm. beneath the costal margin in the right midclavicular line and 15 cm. beneath the tip of the xiphoid. The gallbladder was not remarkable and contained 50 cc. of thin yellow-green fluid. The esophagus and stomach were grossly negative. The entire small bowel was dilated to twice its usual size. The lumen contained a moderate quantity of liquid, gray-white, fecal material. The wall of the small intestine was

2 or 3 times its usual thickness (Fig. 1). This increase appeared to be due solely to changes in the mucosa which was filled by a white, fatty substance resulting in countless granular, friable, frond-like projections. The rugae were prominent. When the mucosa was cut it was possible to express this white, fatty substance. The Peyer's patches were not grossly discernible. The muscle layers and serosa of the small bowel were not remarkable. The lesion ended abruptly at the ileocecal valve and no abnormalities were present in the large bowel.

There were many discrete, firm, gray-white, enlarged mesenteric nodes which averaged 2 cm. in diameter. The cut surfaces of the nodes were pale gray with a few pinhead-sized, discrete nodules scattered throughout.

The kidneys weighed 340 gm. There was a 2.5 cm. shallow depression with a firm, gray-yellow center and thin, dark purple periphery at the lower pole of the right kidney. Adjacent to this area were 15 to 20 small areas of purple discoloration varying from 0.05 to 0.5 cm. in diameter.

Gross examination of the remainder of the organs showed nothing of note.

Microscopic Examination

The esophagus, stomach, and duodenum were negative. Identical histologic lesions were present in the jejunum and ileum. The villi varied greatly in size (Fig. 2), some being three or four times the usual width, while slender villi were frequently five or six times the usual length. This increase in size was associated with the presence of a lipoid substance or substances in three physical forms. The first was a homogeneous, lightly stained, fine coagulum which took a pale pink stain with phloxine, and distended the lacteals of the enlarged villi (Fig. 3). The second form appeared as vacuoles of various sizes scattered throughout the coagulum and also making up a small part of the area of the villus. The third consisted of accumulations of large mononuclear cells with vacuolated or foamy cytoplasm scattered throughout the villi. The tunica propria, present at the periphery of the dilated villi, was thickened, and there was increased cellularity. There were large, dilated spaces throughout the submucosa containing the same elements noted in the villi. The muscularis and serosa were normal at low power magnification.

At higher magnification the large spaces in the submucosa containing the pink coagulum were seen to be lined by endothelial cells. Some of these endothelial cells were enlarged and contained small vacuoles in the cytoplasm. The presumption was that these large spaces were lymphatic sinusoids. A majority of the sinusoids contained many mononuclear cells which varied in size from that of the usual phagocyte

to gigantic "foam" cells eight to ten times the diameter of the former (Fig. 4). These large cells had an indistinct, small nucleus and very large, multivacuolated, frothy cytoplasm. They commonly occurred about the periphery of the dilated lymphatics, and in many villi they seemed to have infiltrated into the lipoid substance and to be phagocytizing it. In some sinusoids they occupied most of the area, but generally they made up a small portion of the substance. Multinucleated giant cells were occasionally seen and these had foamy cytoplasm. Also enmeshed in the lipoid material of the villi were a few lymphocytes and plasma cells. The interstices of the displaced tunica propria showed considerable pink coagulum, vacuolization, and infiltration with mononuclear cells with the foamy cytoplasm. There was an increase in the number of lymphocytes, plasma cells, and connective tissue in the tunica.

The epithelium of the mucous membrane of the villi was intact and only a rare cell contained a few vacuoles in its cytoplasm. The cells of the crypts of Lieberkühn were similarly free of change, as were the goblet cells and the lymphoid follicles.

The stroma of the mucous membrane was distended by many large, clear vacuoles and much pink coagulum, and there were increased eosinophils, plasma cells, and lymphocytes present. Diffusely scattered throughout the stroma were many large mononuclear cells with frothy cytoplasm similar to those seen in the villi. In some sections, particularly those stained for fat, the lipoid could be seen distending the villi and extending throughout the tunica and muscularis to branch off sharply at right angles in the submucosa and run parallel with the muscular layers, nicely outlining the lymphatic pattern.

The muscular layers showed only scattered foci of a few lipoid-bearing cells. These were more common in the connective tissue between the circular and longitudinal muscles. In the serosa there were a few areas in which a moderate number of mononuclear cells and an occasional large area contained material similar to that in the villi, giving a positive reaction to the fat stain. The endothelial cells of a blood vessel occasionally contained vacuolated cytoplasm, also giving a positive reaction for fat.

The large intestine was negative.

When the jejunum and ileum were stained with sudan IV the lipoid material became bright red. These lipoids were pink-red with Nile blue sulfate (neutral fats). Polariscopic examination showed a few small, doubly refractile pinpoints scattered throughout the villi, mucosa, and submucosa. Most of the lipoid substance, however, was nonrefractile and no more than 10 per cent could be considered to be anisotropic.

Chemical analysis of a section of small intestine * showed the fatty substance in the analyzed specimen to be mainly phospholipids and neutral fat. The cholesterol was only slightly increased (Table I).

As a result of differential staining, polariscopic examination, and chemical analysis, the lipoid material was considered to be a mixture of both phospholipid and neutral fat with possibly a very slight increase in cholesterol. Neutral fat predominated as evidenced by the Nile blue sulfate stain and chemical analysis.

The cortical and medullary sinuses of the mesenteric lymph nodes were dilated to four or five times their usual size and filled with a pale, homogeneous coagulum similar to that seen in the villi (Fig. 5). There was crowding and thinning out of the follicles. As in the villi, the coagulum was accompanied by many mononuclear cells with foamy

TABLE I

Results of Analysis of Small Intestine

Wet weight of tissue used	3.2 gm.
Dry weight of tissue used	0.4886 gm
	Mg. per 100 mg. dried tissue
Total cholesterol	3.16
Total phospholipids	14.95
Total fatty acids	19.9

cytoplasm varying in size from the usual phagocyte to cells eight to ten times the diameter of the former (Fig. 6). There was moderate increase in trabecular connective tissue. Phagocytes which had engulfed hemosiderin were found in all nodes examined.

In many of the nodes, huge mononuclear cells with frothy cytoplasm and vesicular nuclei replaced the coagulum in the dilated sinusoids. Scattered throughout sinusoids of all the nodes were many lymphocytes, plasma cells, and mononuclear cells. Many of the endothelial cells lining the sinusoids were swollen and had a vacuolated cytoplasm. Occasionally multinucleated giant cells were found.

In the lymph nodes many cells contained circular, discrete, acidophilic intranuclear inclusions that had at their periphery a clear, thin zone which was surrounded by fragmented nuclear chromatin. Rarely a similar inclusion was present in the cytoplasm of a cell. The intranuclear inclusions were found in plasma cells and in the nuclei of both the large and small macrophages which contained lipid. While the lipid in the coagulum and cytoplasm gave a positive stain for fat and was apparently the same as the fat in the small bowel, none of the nuclear

* We are indebted to Dr. S. J. Tannhauser for the analysis of this specimen.

or cytoplasmic inclusions gave a positive stain for fat and under the polariscope they were not anisotropic.

A large lymph node taken from the junction of the cystic and common ducts showed similar changes but to a lesser degree. The lymphoid sinuses were dilated to about twice their usual width and contained many phagocytes with foamy cytoplasm. There was only a small amount of coagulum present and there was only a slight encroachment upon the lymphoid elements. There was a slight increase of trabecular connective tissue throughout.

There was much granular, black pigment present in the tracheal nodes together with fibrosis. Phagocytes with foamy cytoplasm were occasionally found.

Microscopic sections of the heart were not remarkable. Alternating areas of atelectasis and emphysema were present in the lungs, and there were foci of alveoli filled with edema fluid and red blood cells.

Microscopic examination of the splenculus showed the usual splenic structure. The changes found in the spleen were not present.

The liver structure was well preserved. The sinusoids were slightly dilated by red cells. There were scattered foci of collagen surrounded by lymphocytes, plasma cells, and large mononuclear cells with vesicular nuclei and large, pale pink, homogeneous cytoplasm. Occasionally, fibroblasts could be identified in the centers of these lesions which frequently, for the most part, were periportal in distribution but were occasionally found in midzonal or pericentral areas. Many liver cells contained hemosiderin and frequently this substance was present in the large mononuclear cells in the granulomatous lesions.

A small area of old infarction was present in the cortex of the kidney. The glomeruli, tubules, interstitial tissue, and pelvis were negative except for small granules of hemosiderin in the epithelium of many of the collecting tubules.

There was a large focus of necrosis in the medulla of the adrenal, surrounded by phagocytes with vacuolated cytoplasm, lymphocytes, and plasma cells. In the cortex were a few areas of collagen in which were seen phagocytes, and there was one large area in the cortex that was composed entirely of huge phagocytes with a foamy cytoplasm.

The bone marrow was very cellular, with hyperplasia and maturation of both the erythrocytic and granulocytic series. Megakaryocytes were present in usual numbers. A few intranuclear inclusion bodies in plasma cells, similar to those seen in the mesenteric nodes, were present. There were large phagocytes containing hemosiderin. There was no evidence of leukemia.

Anatomical Diagnoses. Intestinal lipodystrophy (Whipple's dis-

ease); focal granulomatous lesions of the liver; intranuclear inclusion bodies in plasma cells, cause unknown; focal necrosis of the adrenal; healed infarct of kidney; surgical absence of spleen; healed left rectus incision.

COMMENT

The blood findings in this case were bizarre. A persistent leukocytosis of 20,000 to 50,000 white blood cells per cmm. was present for months during the early part of the patient's illness. There was relative and absolute lymphocytosis with 70 to 80 per cent of the white cells composed of "large," "atypical," or "abnormal" cells of the lymphoid series. Most of these cells were large with a clear, light blue cytoplasm. It was noted that many of these cells had nuclei of a "young" type and nucleoli were sometimes present.

Following splenectomy, the absolute as well as the relative number of lymphocytes decreased to within normal limits and remained normal for approximately 1 month. The lymphocytes appeared normal. During this period there were many toxic polymorphonuclear neutrophils and many young forms of polymorphonuclear cells. The number of lymphocytes gradually increased to 30,000 and ranged between 15,000 and 20,000 during the last month of life. During this period the cells could not be classified. In general, the cells were younger than when first seen, as indicated by the deep blue of the cytoplasm of many of them. Some of these were true blasts with nucleoli; some resembled young lymphocytes and some young monocytes. The platelets were maintained at a higher level following splenectomy.

At autopsy no anatomical evidence for a diagnosis of lymphatic leukemia was found. Reinhart and Wilson[4] described a "benign lymphocytosis" in their case with a white cell count varying from 15,000 to 20,000, but gave no further data regarding the blood picture. The concomitant occurrence of lymphocytosis and acute hemolytic jaundice is unusual. Acute hemolytic anemia is frequently accompanied by leukocytosis, but it is generally of the myeloid series. Lymphocytosis is stated to occur in chronic diseases where there is a stimulation to reticular hyperplasia such as in lues or tuberculosis. No evidence of any of these infectious diseases was found in our case. It was felt that in the present case the blood showed an unusual type of lymphocytic or histiocytic response of unknown causation.

The erythrocytes were described as showing "normal fragility" in the early part of 1942 at another hospital, but in March, 1942, when studied at the Thorndike Memorial Laboratory of the Boston City Hospital, an increased fragility to the action of hypotonic saline was demonstrated. Other findings consistent with hemolytic jaundice, such

as increased urobilinogenuria, hyperbilirubinemia, splenomegaly, anemia, spherocytosis, and reticulocytosis (27 per cent), were found. Histologic examination of the excised spleen showed findings consistent with hemolytic jaundice. Three brothers, two sisters, and one daughter of the patient all had normal erythrocytic fragility curves.

The anemia which occurs in Whipple's disease has never been adequately explained. It is usually described as "hypochromic" or "secondary." The present case is the only case on record in which fragility studies were made. In this connection it is of interest to consider the effects of lipoid substances upon hemolysis of red blood cells. Haden,[11] Castle and Daland,[12] and others have shown that sphericity of the red blood cells is associated with increased fragility. Tompkins [13] was able, by repeated intravenous injections of lecithin, to produce in rabbits a slowly progressive hemolytic anemia associated with a slight increase in hypotonic fragility and a slight tendency of the erythrocytes towards sphericity. Ponder [14,15] has shown that erythrocytes become spheroidal in suspensions of lecithin. There is some experimental evidence which supports the thesis that phospholipids in circulation are adsorbed on erythrocytes. Bloor [16] and Freeman and Johnson [17] have shown the presence of a hemolytic agent in lymph from the thoracic duct during the adsorption of ingested fat. The hemolytic agent responsible is probably a combination of free fatty acid and soap. In view of the fact that the above various lipoid substances have been shown to have a hemolytic action, it would seem that study of the hemolytic action of the lipoid substance found in Whipple's disease would be indicated, since it is possible that such studies might lead to the explanation of the anemia in these cases.

Eosinophilic inclusion bodies were seen in the nuclei of plasma cells in the mesenteric nodes. These were quite striking and have not been noted before in this laboratory, nor have they been described before in this disease.

Discussion

The average age for this group of patients is 49 years, the youngest reported being 36 years of age, while the oldest was 74 years. The greater number occurred in the third and fourth decades. Seven of the 8 cases occurred in males.

The usual symptoms are progressive loss of weight and strength terminating in marked emaciation and diarrhea. Five of the 8 patients had suffered from diarrhea for periods varying from a "brief episode" to 2 years, with this symptom in a majority of the cases persisting for a few months before demise. In one case [4] "constipation" was reported. The stools have been described generally as light yellow, creamy, or

clay-colored except for one case in which the stools were dark brown to black and were guaiac-positive. Some stools have contained bile while others were bile-free. Chemical analyses of stools from 2 cases are available. In Whipple's case [1] 80 per cent of the dried stool was fat, of which 50 per cent was neutral fat and 30 per cent fatty acids. In the case reported by Pearse,[10] 60 per cent of the dried stool was fat, of which 44 per cent was fatty acids, 12 per cent neutral fat, and 3 per cent was unsaponifiable. These results should be compared with Fowweather's [18] figures for normal stools in which 17.5 per cent of the dried stool is fat, of which 7.31 per cent is neutral fat, 4.6 per cent soap fat, and 5.64 per cent free fatty acids. After treatment of Pearse's patient the lipoid content of dry matter decreased from 60 per cent to 25 per cent and only fatty acids were present. Thus in both cases the total fat of the stools was increased; in one case fatty acids were in excess and in the other neutral fats predominated.

No parasites nor ova were seen in any case, nor were any significant organisms cultured from the stools.

In those cases in which agglutination tests for the typhoid, paratyphoid, and dysentery groups were done, the results were negative.

Patients have complained of abdominal distention, vague discomfort, particularly over the upper abdomen, crampy pains over any portion of the abdomen, and even colicky right upper quadrant pains suggesting acute cholecystitis. In 5 of the reported cases there was a long history of polyarthritis varying from 5 to 15 years, and in 4 of 7 cases in which the thorax was examined there was an organizing or organized pericarditis which was described as "obliterative" or adhesive; 3 showed pleural adhesions and 3 revealed healed verrucous endocarditis.

Progressive anemia generally described as "secondary" has been described in most cases. One case (Blumgart [2]) had a color index greater than 1 and ours showed increased fragility consistent with a diagnosis of hemolytic anemia. White, differential, and reticulocyte counts have been within normal limits except for the present case and one other discussed above. The urine was negative in all cases.

In 5 cases a slight, yellow, generalized pigmentation of the skin was noted. Interstitial fibrosis of the pancreas was described in 2 of the 7 autopsied cases. In 1 case a granulomatous lesion was present in the liver which was similar to that described in the present case.

The pathologic findings in Whipple's disease are characterized by deposits of fat and fatty acids in the small intestine and mesenteric lymph nodes. Grossly, the mucosa of the jejunum and ileum is swollen and is flecked with minute deposits of yellow-white lipoid substance. The severity of the lesion may vary, the mucosa in one case being pink

flecked by yellow, while in other cases the deposits are so great as apparently to involve the entire mucosa and give to it a definite yellow color. The mucosa is not ulcerated and the Peyer's patches are not unduly prominent. Microscopic examination of the bowel wall shows large deposits of lipoid substance in the villi, submucosa, and the interglandular tissue of the mucosa and submucosa. The villi are double the usual length and breadth and are filled with lipoid substance. These areas also show large spaces lined with endothelial cells which suggest dilated lymph radicles. Lipoid substance completely fills many of these spaces. Scattered throughout the villi, submucosa and mucosa are many mononuclear cells whose cytoplasm is vacuolated. A few multinucleated giant cells containing vacuoles are also present. Deposits of lipoid are found in the endothelial cells lining the capillaries. The lymph follicles are not involved to any striking degree, containing but an occasional fat-laden monocyte. The lipoid substance takes the sudan IV stain well, the coagulum, vacuoles and foamy cytoplasm taking a bright red stain. These substances stain pink-red with the Nile blue sulfate stain which is considered suggestive of neutral fat. Polariscopic examination shows most of the substance to be non-doubly refractile. Chemical analysis indicates that the lipoid substance is largely phospholipids and neutral fat. Whipple's analysis showed the neutral fat to have a low saponification number, which he interpreted as indicating either some abnormality of the fat or the presence of some nonsaponifiable substance mixed with it.

The mesenteric and occasionally the retroperitoneal and peripancreatic lymph nodes are enlarged and their cut surfaces are pale yellow. Microscopic sections of the nodes show that the cortical and medullary sinuses are markedly dilated and are filled with lipoid substance similar to that noted in the small intestine. In many nodes the follicles have been compressed or have disappeared. As in the intestine, there are many mononuclear cells containing the lipoid substance. Other sinuses are filled with huge, lipid-laden mononuclear cells.

The nature of the changes in the joints in Whipple's disease is unknown as the joints were not studied in any of the cases reported.

The cause of Whipple's disease is unknown. Obstruction of the thoracic duct or mesenteric lymph vessels comes naturally to mind. However, in cases of chylous ascites due to obstruction of the thoracic duct by various causes no enlargement of mesenteric nodes nor intestinal lesions such as are described in cases of Whipple's disease occur. Also, Karoliny [19] was unable to reproduce the lesions by ligation or compression of the thoracic duct in animals. Furthermore, no evidence

of occlusion of the lymph passages or thoracic duct has been found in any of the reported cases of this disease.

The clinical course is not unlike that of nontropical sprue. However, it has never been demonstrated that any characteristic pathologic lesions occur in the intestines in sprue.[20] Certainly, no lesions have ever been demonstrated in sprue that resemble those of Whipple's disease.

Idiopathic intestinal lipodystrophy does not fit into any of the known diseases of lipoid storage such as Niemann-Pick's disease, Hand-Schüller-Christian's disease, or Gaucher's disease. All anatomical, clinical, and chemical evidence serves to exclude Whipple's disease from this group. Such lipoid diseases occur primarily in infants or in the first decades of life. The lipoid deposits are widespread throughout the reticulo-endothelial system, viscera, and bones, and in Gaucher's disease the lipoid stored is a cerebroside, in Niemann-Pick's disease, a phospholipid, and in Hand-Schüller-Christian's disease, cholesterol and cholesterol esters. In Whipple's disease all reported cases have occurred in middle-aged or elderly subjects. The lesions are limited to the small intestine and the associated mesenteric nodes, and the lipid for the most part is neutral fat.

Localized deposits of lipid have been described in the gastric and intestinal mucosa in middle-aged and elderly persons.[21,22] These lesions differ from those of Whipple's disease since they are made up of small groups of cells containing fat droplets and there is no giant cell formation. Clinically, no symptoms have been attributed to these lesions.

Whipple[1] suggested that the fat is abnormal in some way. The evidence of this, in his opinion, was the low saponification number and the presence of the peculiar foreign body giant cell reaction to the fat. He also emphasized the presence of numerous ecchymoses and blood pigment as suggesting some action on capillary walls. In addition, Whipple pointed out that the pathologic changes are limited to the structures concerned with fat absorption while there is no involvement of other lymphatic tissue in the body.

Pearse's[10] patient improved remarkably on the prolonged daily administration of bile salts. Under this regime the symptoms disappeared, vitamin A absorption improved, and the fat content of the stools approached normal. Upon purposeful omission of the medication, the patient had two successive remissions with nausea, indigestion, flatus, and diarrhea as chief symptoms. These symptoms were relieved both times by return to the regime of daily bile salts and the patient was well 1 to 2 years after operation. This remarkable recovery of Pearse's patient on bile salts is hard to explain in view of current theories of fat metab-

olism. Pearse merely suggested that some obscure qualitative factor of bile or bile salts is lacking and that replacement of this unknown factor effects the cure.

SUMMARY

1. The eighth reported case of Whipple's disease, or intestinal lipodystrophy, is described. This case is of particular interest because of the bizarre hematologic picture that caused it to be confused with lymphatic leukemia and hemolytic icterus.

2. Whipple's disease is characterized pathologically by the deposit of lipids in the mucosa of the small intestine and in the mesenteric lymph nodes. Clinically, the disease is marked by asthenia, anemia, arthritis, steatorrhea, abdominal distention and discomfort, and usually progresses to a fatal termination.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

Plate 188

Fig. 1. Small intestine, showing the mucosa containing the lipoid substance.

Fig. 2. Low-power view of the small intestine illustrating the marked variation and increase in size of the villi due to the lipoid substance. Phloxine-methylene blue stain. $\times$ 55.

1

2

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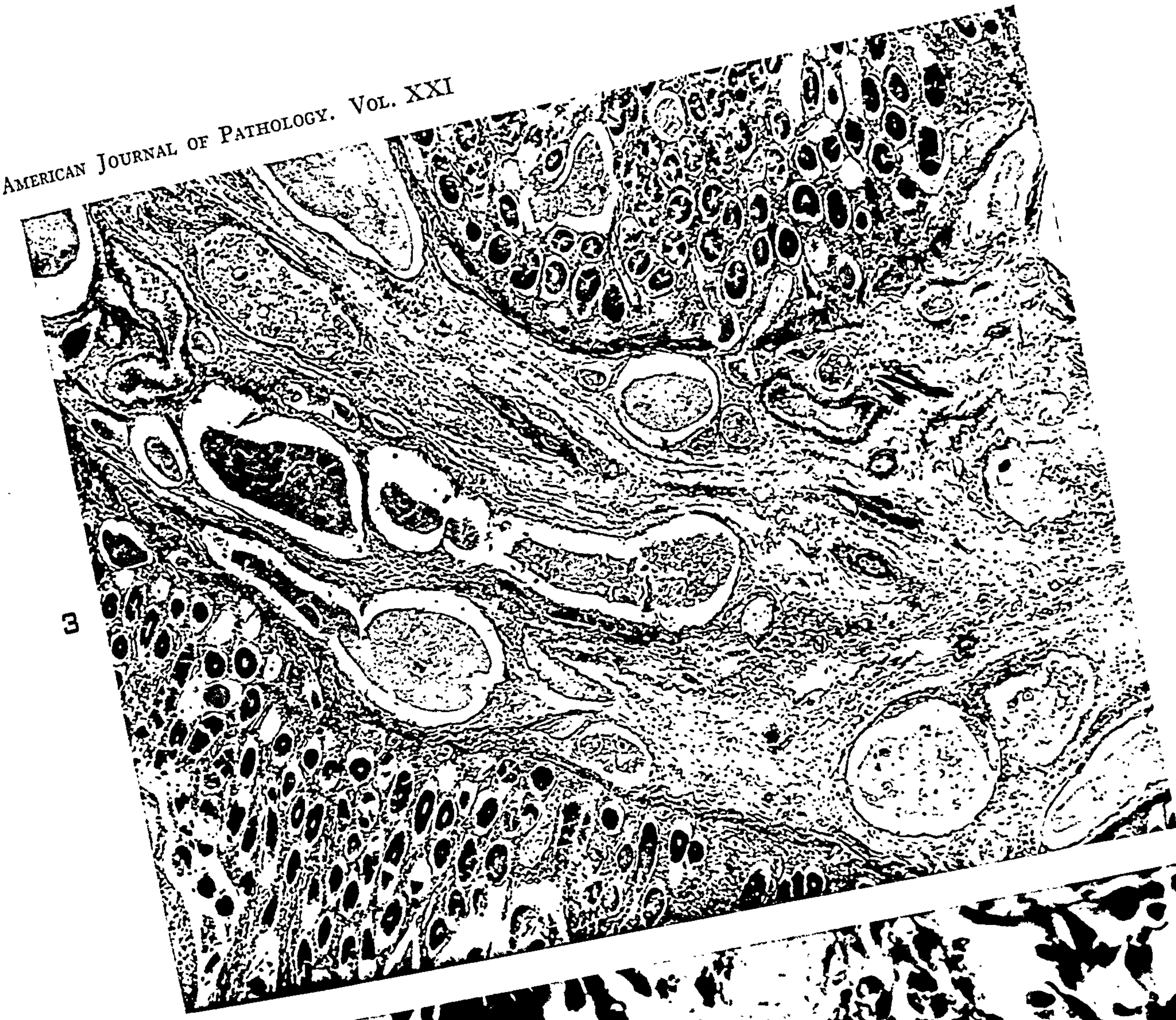
Plate 189

Fig. 3. Low-power view of the small intestine showing the lacteals of the enlarged villi distended by lipoid. Phloxine-methylene blue stain. × 130.

Fig. 4. Section of the small bowel showing mononuclear cells containing the lipoid substance. Phloxine-methylene blue stain. × 260.

3



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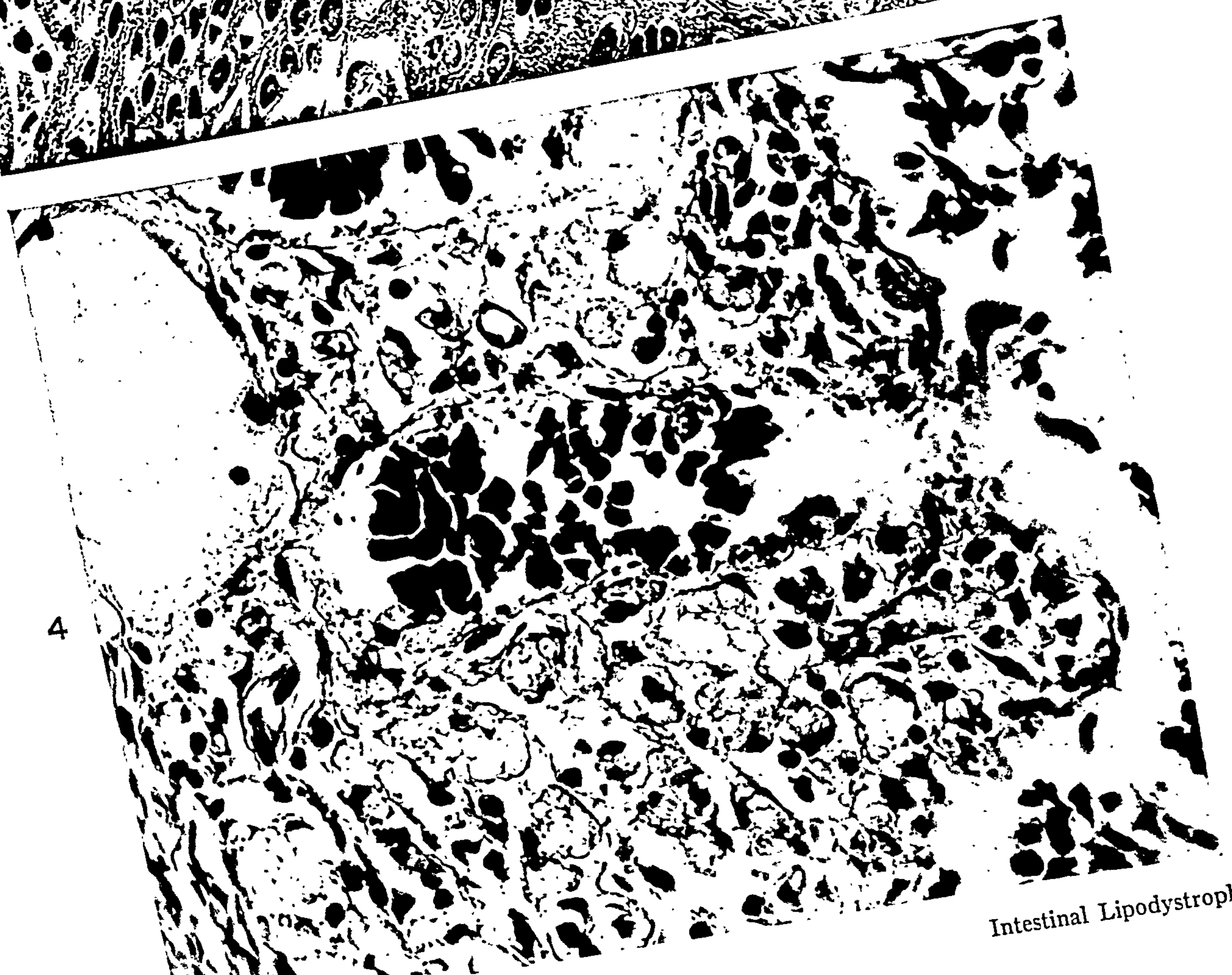

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PLATE 190

FIG. 5. Low-power view of a mesenteric lymph node showing the distention of the cortical and medullary sinuses. Phloxine-methylene blue stain. $\times$ 130.

FIG. 6. Section of a mesenteric lymph node showing the lipoid-laden mononuclear cells. Phloxine-methylene blue stain. $\times$ 215.

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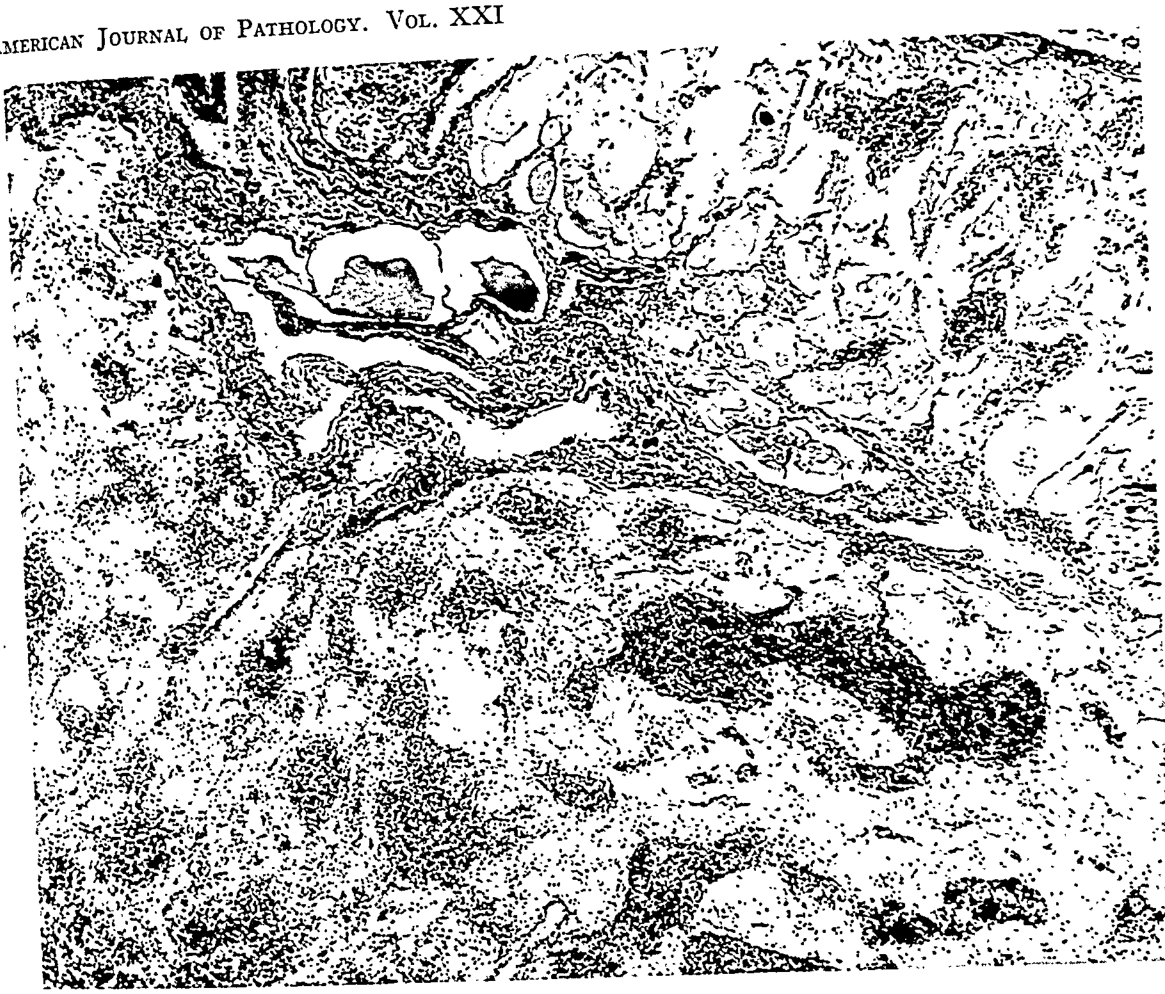

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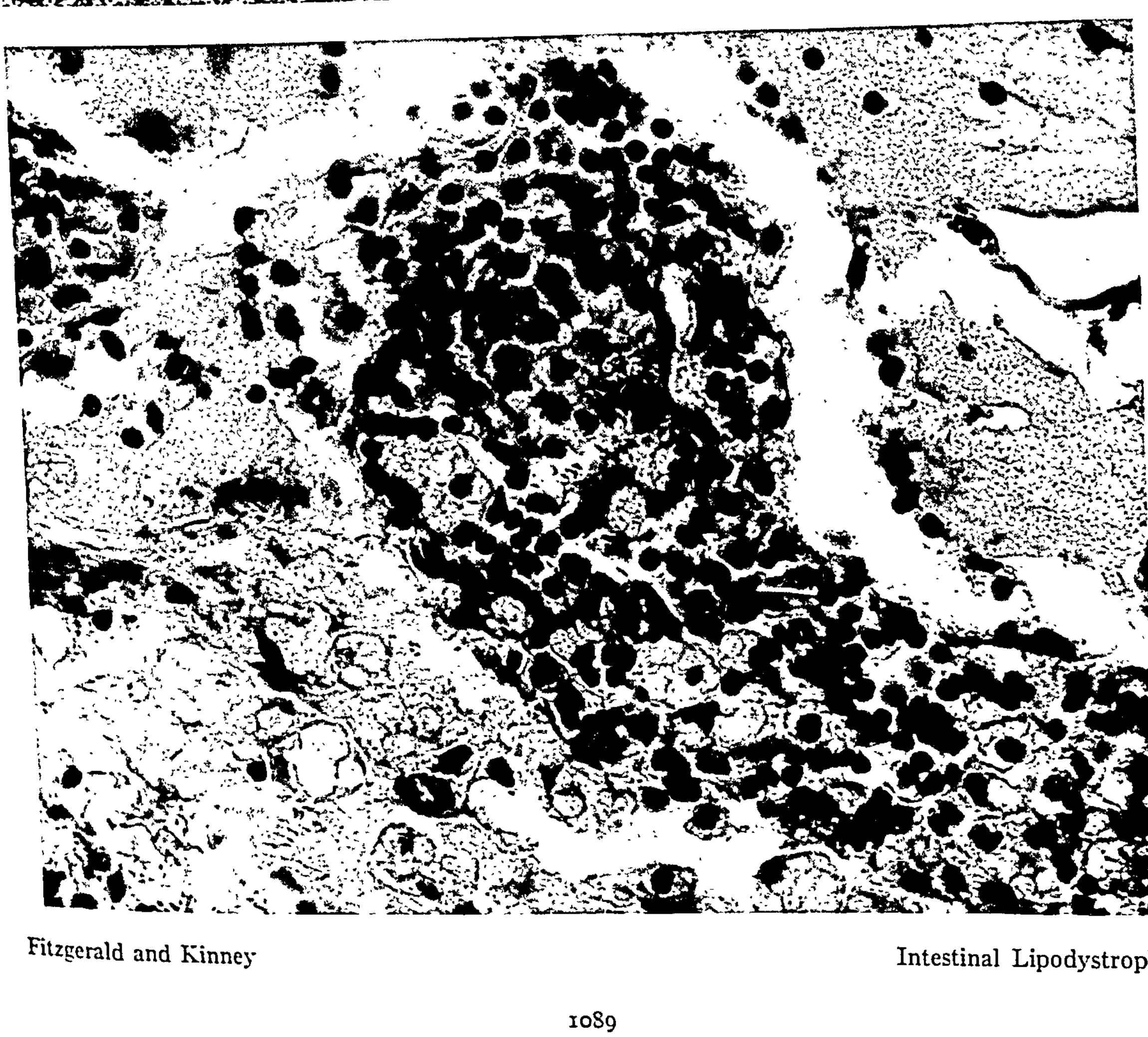

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Intestinal Lipodystrophy

A PATHOLOGICAL STUDY OF RENAL DAMAGE PRODUCED BY SULFADIAZINE IN RATS

Development, Repair, and Residua *

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Since the introduction of sulfonamide drugs as therapeutic agents, there have been numerous reports of renal complications. A fairly complete bibliography of the reported lesions in fatal cases is contained in the paper of Murphy, Kuzma, Polley, and Grill.[1] Studies in various experimental animals have also been described.[2-12] In most of these, renal lesions have been produced by a single dose or a few overwhelming doses of one of the sulfonamides. No extensive studies have been reported concerning the phases of recovery.

The present study was made in connection with a physiological study of the production and prevention of renal lesions. This report will present observations on the morphological aspects of the development, early and late recovery stages, and residual effects of renal damage produced in rats by sulfadiazine. The physiological aspects of the study are presented elsewhere.[13]

Methods

The development of renal lesions was studied in over 200 albino rats which were placed on diet no. 803 [13] within a week after weaning. This diet consisted of 10 per cent casein, 8 per cent Crisco,† 77 per cent sucrose, and 1 per cent sulfadiazine with appropriate salt and vitamin supplements. At intervals varying from 1 to 30 days after beginning the diet, rats died or were killed and the tissues were fixed in 3.7 per cent aqueous formaldehyde, embedded in paraffin, sectioned, and stained with hemalum-eosin-azure and van Gieson's picrofuchsin.

For the purpose of studying the recovery stages, 12 weanling rats were given diet no. 803 for 30 days. Then, under ether anesthesia, the right kidney was inspected. All 12 rats showed gross evidence of marked renal damage. In 7 rats the right kidney was removed for study. The rats were then given diet no. 819 [13] which differed from diet no. 803 only in that the sulfadiazine was replaced by an equal weight of sucrose. The rats died or were killed after 2 to 15 days and the tissues were examined as before.

For the purpose of studying residual renal lesions, 16 rats were

* Received for publication, December 11, 1944.

† Crisco is a partially hydrogenated vegetable oil made by Procter and Gamble, Cincinnati, Ohio.

given diet no. 803 to produce marked renal damage and the right kidney was then removed for study. After nephrectomy the diet was changed to no. 819 (without sulfadiazine) and the rats were killed 3 months later and studied as before.

Observations

The earliest lesions were in the renal papillae and consisted of a chalky striation. The surface of the kidney appeared normal. In rats killed after longer intervals, the chalky striation extended into the cortex, and in most rats surviving 30 days the striation reached from the tip of the papilla to the capsule, following the course of the collecting tubules. The kidneys in these rats were enlarged and pale tan, and were covered with a fine, chalky white stippling.

Inspection of the stained sections showed a striking dilatation of the tubules, visible without magnification (Figs. 1 to 5). This dilatation was more or less restricted to that portion of the kidney which showed the chalky striation grossly. In sections prepared by fixing the kidneys in formaldehyde vapor,[14] cutting frozen sections, transferring them directly to dry slides, and mounting in xylol-clarite, many tubules were filled with crystals and amorphous débris. Most of the crystals were identified by their characteristic shape as acetylsulfadiazine. In ordinary paraffin sections, the crystals were not seen.

The tubular dilatation was noted first in the papilla and later extended to involve the distal convoluted tubules and ascending limb of Henle's loop. In a few kidneys the dilatation involved the entire nephron, including the glomerular capsule. Many of the dilated tubules showed only a flattening of the epithelium. In others, the epithelium was basophilic and showed an increase in number of nuclei or nuclear pyknosis. Epithelial necrosis was encountered very rarely. The epithelium of the dilated tubules rarely contained fat, but basal, fine, fat droplets were demonstrated occasionally by fat stains of frozen sections in the nondilated proximal convoluted tubules and Henle's loop. Epithelial calcification was rare.

Dilated tubules often contained oxyphilic casts. Many of these were coated with neutrophilic leukocytes. In such casts, fan-shaped, empty spaces representing the spaces once occupied by sulfonamide crystals were often present. In some rats no lesions other than tubular dilatation were noted.

The interstitial reaction varied considerably. In the early stages there was occasionally an infiltration of neutrophils around some of the dilated tubules. Intertubular edema was more common. Later an in-

crease of fibroblasts occurred. Occasionally such fibroblasts were arranged concentrically about a dilated and pus-filled collecting tubule producing a picture suggestive of a small granuloma. In some rats surviving 1 month, slight to moderate fibrosis was seen, chiefly in the form of collars about collecting tubules, but also to a lesser extent as a delicate, diffuse, medullary fibrosis extending occasionally into the cortex.

Lesions of the renal pelvis and ureters occurred rather infrequently and were most common in rats which showed few or no intrarenal lesions. A few minute calcareous concretions or oxyphilic casts containing fan-shaped spaces were noted in a few rats. Several rats showed hydronephrosis attributed to impaction of such débris in the ureters. A few rats showed hyperplasia and squamous metaplasia of the renal pelvic epithelium, but in most rats the renal pelvis and ureters were histologically negative. The urinary bladder was not examined regularly.

In spite of diligent search, lesions of renal blood vessels or glomerular tufts were not encountered.

Repair. In the kidney of one rat killed 2 days after discontinuing sulfadiazine administration there was no gross or microscopical evidence of recovery. In rats killed after 5 days there was definite gross and microscopical evidence of beginning renal recovery which was more complete the longer the animal lived. The gross enlargement and chalky stippling decreased and the kidneys became darker than at the time of operation. The cortical surface developed a fine pitting. Sulfonamide crystals disappeared from the tubules. Histologically, the tubular lumina became free of pus and casts, and the epithelium showed heaping up of nuclei, rather numerous mitoses, and cytoplasmic basophilia. Dilatation decreased and many tubules had a normal appearance. The rate of recovery was variable. One rat had recovered more in 5 days than another had in 11 days. However, in all rats some evidence of damage persisted. Nearly all kidneys showed a slight to moderate fibrosis in the medulla and most showed a partial destruction of a number of nephrons.

This destruction of nephrons was most apparent in the subcapsular area where distal convoluted tubules had collapsed and atrophied. This resulted in a decrease of thickness of the supraglomerular zone so that glomeruli came to lie against the capsule. In areas of tubular destruction, a slight fibrosis and lymphocytic infiltration was observed occasionally.

Residual Lesions. All 18 rats surviving 3 months after cessation of sulfadiazine administration showed residual renal damage. Grossly,

the kidneys were normal in color but showed a nodular surface with many small retracted scars and an adherent capsule. They cut with increased resistance.

Microscopically, there was focal to diffuse absence of the supraglomerular zone of convoluted tubules. In these areas of cortical atrophy, numerous shrunken but otherwise normal glomeruli lay side by side without the normal complement of intervening tubules. Such areas showed fibrosis, lymphocytic infiltration, and scattered remnants of tubules. Often, at least one-half of the glomeruli visible in the sections were located in such scars. Usually a broad band of fibrous tissue extended from such cortical scars into the medulla. In the band of scar tissue there were normal and atrophic collecting tubules. The inner cortex and medulla showed moderate to marked focal to diffuse fibrosis with an apparent decrease in the number of collecting tubules. No definite vascular lesions were noted. In the portions of the cortex not involved in atrophy and scarring there was hypertrophy of glomeruli and tubules.

Discussion

The mechanism of the renal damage produced by sulfonamides is unknown. Most authors attribute the tubular lesions to mechanical irritation of the epithelium by the sharp crystals of sulfonamide precipitated there. Some authors, however, suggest that the sulfonamides exert a direct toxic effect on the epithelium of the tubules. In the present study there is some evidence which supports the latter hypothesis. Some kidneys showed massive deposition of sulfonamide crystals in the tubules with no epithelial reaction while other kidneys showed relatively slight deposition of crystals with marked epithelial degeneration and proliferation.

The absence of glomerular and other renal vascular lesions in this series of over 200 rats with marked renal damage indicates that, in the rat, these structures are not particularly susceptible to injury by sulfadiazine. The extreme infrequency of epithelial calcification in this series is also of interest in view of the fact that it is reported to have occurred regularly under different experimental conditions in other laboratories.

The renal lesions encountered in the present study and those reported by others in rats, dogs, monkeys, mice, and man are all quite similar. The collecting and distal convoluted tubules appear to be the chief sites of damage. In the rat, we have shown that permanent renal damage can result from sulfadiazine administration. It is not unlikely that similar residual lesions might be found in other experimental animals and in man if a search were made.

Summary

The renal lesions which develop in rats given a low casein diet containing 1 per cent sulfadiazine[13] have been studied histologically. These lesions appear first in the renal papillae and extend later into the cortex. There is a deposition of sulfonamide-protein casts in the tubules accompanied by dilatation of the tubules, degeneration and proliferation of tubular epithelium, and leukocytic exudation. Interstitial edema, leukocytic infiltration, and fibrosis are common. Grossly, the kidneys are enlarged, pale, and stippled with chalky white markings. The cut surface shows chalky radial striations extending out from the papilla.

Withdrawal of the drug after the development of marked renal lesions results in partial recovery. Some damaged tubules recover completely while others undergo atrophy and disappear. Kidneys examined 100 days later are nodular and scarred, and show destruction of the distal convoluted tubules of as many as one-half of the nephrons. There is prominent fibrosis.

The possible occurrence of similar permanent renal damage in other experimental animals and in man is suggested.

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DESCRIPTION OF PLATES

Plate 191

Fig. 1. Marked dilatation of renal tubules. Hematoxylin-eosin-azure stain. × 4.

Fig. 2. Normal kidney for comparison. × 4.

Fig. 3. Collecting tubules showing cellular and protein-sulfonamide casts. Spaces are left in cast after sulfonamide is dissolved. × 170.

Fig. 4. Collecting tubule containing pus and showing epithelial degeneration. There is also an interstitial leukocytic infiltration. × 340.

Fig. 5. Collecting tubules showing increased number of nuclei. × 340.

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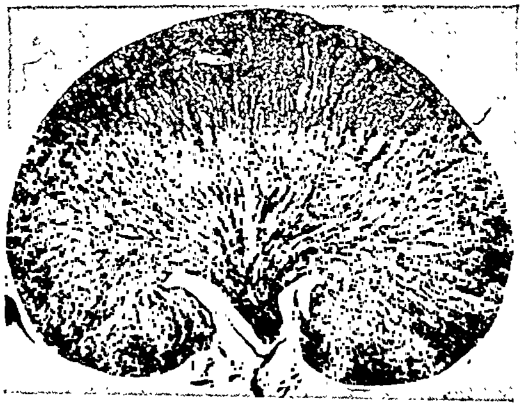

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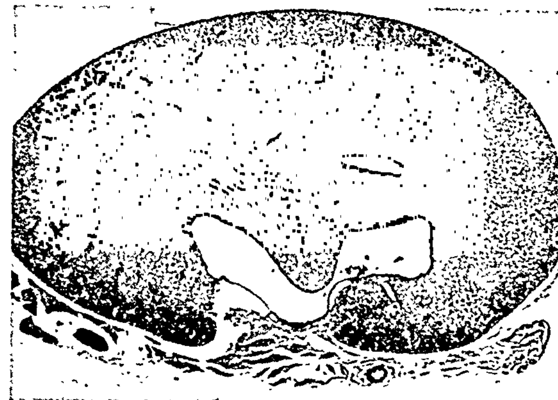

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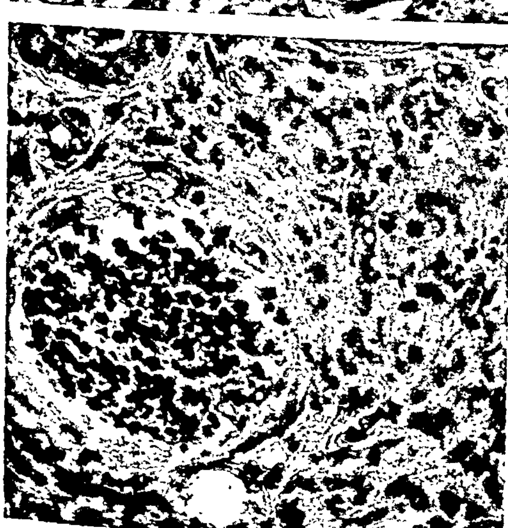

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Endicott and Kornberg

Renal Damage Produced by Sulfadiazine

PLATE 192

FIG. 6. From left to right: normal kidney, enlarged stippled kidney after 30 days of sulfadiazine administration, and nodular scarred kidney 3 months after cessation of sulfadiazine administration. × 1.

FIG. 7. Cortical scar from a nodular kidney, showing lymphocytic infiltration. agglomeration of glomeruli, and atrophy of tubules. Van Gieson stain. × 250.

FIG. 8. Cortical scar. × 250.

FIG. 9. Atrophy and collapse of supraglomerular tubules. × 250.

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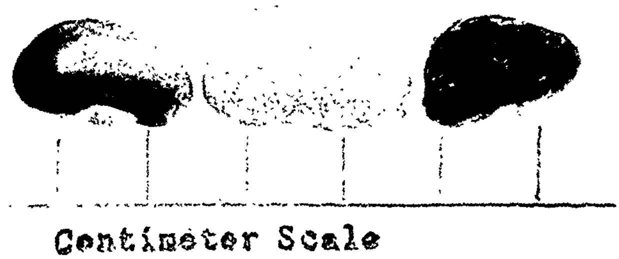

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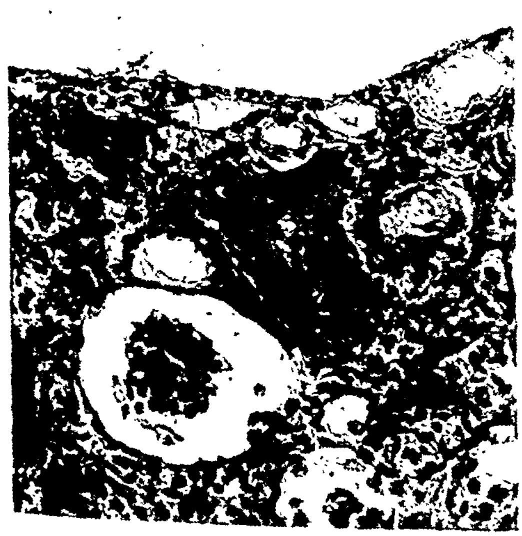

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Endicott and Kornberg

Renal Damage Produced by Sulfadiazine

STUDIES IN VITRO ON THE PHYSIOLOGY OF CELLS

Histologic Reactions of Living Tissues to Hypotonic Solutions *

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A previous study [1] demonstrated, by the method of unstained cell counts, that cells differed in their reaction to distilled water. Testicular cells in suspension were found to be quite sensitive to the lethal and lytic action of this hypotonic fluid. Thymic cells were not as susceptible and suspensions of these cells developed a gelatinous precipitate. No precipitate, however, was observed in suspensions derived from the spleen.

The studies on the effect of distilled water have been continued by exposing freshly excised viable tissues to hypotonic and isotonic solutions and then preparing histologic sections of the tissue. By means of this deferred histologic method, it was possible to demonstrate marked differences in the physiologic reaction of cells to distilled water.

The objectives of the present work were: (1) to check the previous findings [1] on the effect of hypotonic solutions on cells in suspension; (2) to investigate the utility of the deferred histologic method as a means of studying the physiology of excised tissues; and (3) to obtain additional data on the reactions of tissues to distilled water and to Ringer's and isotonic sucrose solutions.

Methods

Thymus, testis, and other organs of the rat and rabbit were cut with a sharp knife into pieces measuring 2 to 5 mm. in length. Each piece of tissue was placed in a 100 by 13 mm. test tube which contained 5 cc. of fluid and which was stoppered with a black rubber stopper. The tubes were placed in a perforated metal box and rotated at 80 r.p.m. in a water bath maintained at 2°, 38°, or 45°C. After incubation, the gross appearance of the tissue and of the fluid was noted. The tissue was then fixed in Bouin's or Zenker's solution and paraffin sections were prepared.

The fluids used in these experiments were distilled water, Ringer's solution, and 0.33 molar sucrose solutions, to each of which was added 5 per cent of a phosphate buffer (1/15 molar) having a pH of 7.6.

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Thymus

To study the initial reactions, thymic tissue was exposed to phosphate-water at approximately 2°C. for 30 minutes and then fixed, cut, and stained. In the peripheral zone of the section, the small cortical cells were separated by clear spaces evidently due to interstitial edema (Fig. 1). The cells were round or oval and were normal in size or slightly enlarged. The nuclei stained intensely and some of them had the shape of a horseshoe or of a ring which surrounded a small amount of central cytoplasm. Reticular cells close to the periphery were considerably enlarged (Fig. 2), with well defined cell walls and widely spaced, fine granules in the cytoplasm. The lightly staining nuclei of these cells were also enlarged and had conspicuous nuclear membranes and prominent nucleoli. The histologic changes suggested that these reticular cells had been killed by the distilled water and were undergoing lysis.

In a few areas near the periphery of the section, the cellular structure of the treated thymus was partly replaced by sheaths of thin, basophilic fibrils which formed whorl-like loops around small acinus-like structures (Fig. 3). The ends of the loops were frayed and pointed towards the periphery of the section. The sheaths of fibrils were somewhat loose, and intermixed with the fibrils were round and spindle-shaped thymic cells in moderate numbers. The nuclei of some of these cells appeared elongated.

The acinus-like structure at the center of the whorl of fibrils had, usually, a small, clear space surrounded by two to four flat or cuboidal cells (Fig. 3). The nuclei were moderate in size and stained deeply and uniformly. The cytoplasm was abundant and stained deeply with eosin. These structures were probably Hassall's corpuscles.

More prolonged incubation of thymic tissue with phosphate-water (3 hours at 2°C.) resulted in a marked increase in the number of basophilic fibrils (Fig. 5). The sheaths of fibrils were numerous, thick, and dense, and did not take the silver stain for reticulum. The sheaths formed whorls around small acinus-like structures. The cells in these structures were not so well defined as in the previous section but many of the cells still had deeply staining cytoplasm and nuclei. Small thymic cells were sparse in areas having many fibrils but were numerous in other parts of the cortex (Fig. 4). The nuclei of the cortical cells were horseshoe or ring-shaped. The reticular cells were few in number, apparently due to the lysis of most of them. The persisting markedly enlarged reticular cells had large or small nuclei which stained lightly and homogeneously (Fig. 4). A few large, clear spaces were present in the center of the section and probably represented

areas which were originally occupied by reticular cells (Fig. 5). It is to be noted that the small cortical cells and the large reticular cells reacted differently to the phosphate-water.

The early reactions of thymic tissue to hypotonic solutions at 38°C. were studied by exposing the tissue to a mixture of 7 parts of phosphate-water and 3 parts of phosphate-Ringer's solution for 30 minutes. Sections of the tissue (Fig. 6) had a central area composed of fairly normal cells. In the peripheral zone, the cortical cells were normal in size. A few nuclei were horseshoe-shaped as observed in tissue treated with water at 2°C. Most of the nuclei, however, were round or oval and lightly stained. It is probable that these cells were dead at the time of fixation and that the observed changes in the nucleus were in part due to autolysis at 38°C. In this section, no fibrils were seen. The reticular cells in the peripheral zone were markedly enlarged with very large, poorly staining nuclei.

Thymic tissue exposed to phosphate-water at 38°C. for 1 hour had a moderate number of basophilic fibrils (Fig. 7). Some of the fibrils were isolated from each other; others formed sheaths or a loose network. The sheaths of fibrils did not form whorls or loops. In some sections, acellular areas had fine basophilic granules.

Changes were observed in the blood vessels of the thymic tissue treated with distilled water. In early stages some of the blood vessels had a small amount of amorphous débris which represented the remnants of lysed red blood cells. The endothelial cells of capillaries and small arterioles were enlarged and extended into the lumen (Fig. 17). The cell wall was prominent; the cytoplasm was unstained; the nucleus was large and round, had a light bluish, muddy color, and had no definite granules. The smooth muscle cells of the muscularis were not enlarged; the cytoplasm stained intensely; the nuclei were moderately enlarged and also stained deeply.

In one section of thymus exposed to a mixture of phosphate-water (9 parts) and phosphate-Ringer solution (1 part) at 38°C. for 30 minutes, a small vein was cut diagonally (Fig. 8). The nuclei of the endothelial cells were moderately enlarged. There was a layer of subendothelial cells which were markedly enlarged, with edematous unstained cytoplasm and centrally placed, enlarged, round, poorly stained nuclei. These enlarged cells were probably smooth muscle cells.

Thymic tissue exposed for 1 hour to Ringer's or sucrose solution at 2° and at 38°C. showed none of the changes described above and appeared normal. After prolonged incubation (4 hours at 45°C.) the tissue had relatively few cortical and medullary cells (Fig. 9). In some cases, the sections were almost acellular but the fibrous framework

and the blood vessels of the tissue remained intact. It should be noted that, in these experiments, the solution in which the thymic tissue was incubated became turbid after incubation. Smears and sections of the turbid fluid showed intact, well preserved cells. It would seem, then, that the thymic cells were gradually washed out from the tissue on prolonged incubation with Ringer's or sucrose solution.

It may be concluded that incubation of thymic tissue with distilled water caused nuclear changes in the cortical cells, swelling and lysis of reticular cells, production of a basophilic granular and fibrillar precipitate within the tissue, and cytoplasmic and nuclear enlargement of the endothelial cells of blood vessels.

Spleen and Lymph Node

Splenic tissue of the rabbit was exposed to phosphate-water at 2°C. for 6 hours. The capsule was normal but the cells beneath the capsule showed vacuolization of the cytoplasm and enlargement and poor staining of the nuclei. The cut edge of a rat spleen which was exposed to the phosphate-water for 6 hours had a small amount of fibrillar precipitate (Fig. 10). The thin fibrils were usually eosinophilic but in a few areas they were basophilic. In other experiments, no fibrils were seen in the sections. The splenic pulp and the malpighian bodies in the center of the section were fairly normal except for lysis of the red blood cells.

In several experiments, splenic tissue was exposed to phosphate-water at 38°C. The only changes observed in the section were swelling and vacuolization of the cytoplasm of the cells beneath the capsule.

To recapitulate, the histologic changes of splenic tissue treated with phosphate-water consisted chiefly of the enlargement and vacuolization of the subcapsular cells. Occasionally there was also the formation of a small amount of eosinophilic and basophilic precipitate. The changes were not constant in different experiments and were never as striking as those observed in thymic tissue.

A human cervical lymph node was exposed to phosphate-water for 30 minutes at 38°C. (Fig. 11). The architecture was fairly well preserved. Nearly all of the cells in the node, particularly those near the periphery of the section, were moderately or markedly enlarged, with vacuolization of the cytoplasm. Many of the nuclei of the peripheral cells were enlarged and stained poorly. Control tissues exposed to phosphate-Ringer's and phosphate-sucrose solutions at 38°C. for 30 minutes were almost identical in histologic appearance to tissue fixed immediately after excision.

A cervical lymph node of a rabbit was exposed to buffered water at 2°C. for 5 hours. On examination, the sections showed enlargement

of the lymphocytes with clear, unstained cytoplasm and small, deeply staining nuclei (Fig. 12). In addition, a few small, superficial areas had basophilic fibrils forming a loose network. In some experiments exposure of lymph nodes to phosphate-water failed to cause enlargement of the lymphocytes or the formation of fibrils. For example, a small para-aortic node exposed to phosphate-water at 38°C. for 30 minutes was, on histologic section, fairly normal in appearance (Fig. 13). In another lymph node from the cervical region of a rabbit, the only significant change was an intercellular, homogeneous, eosinophilic precipitate in the medúlla.

It is evident that lymph nodes varied in their reaction to phosphate-water. The cause for the variation was not definitely established. The small para-aortic node of the rabbit was normal in control sections and was not affected by incubation with phosphate-water. On the other hand, the human cervical lymph node was large and firm and showed considerable inflammatory and hyperplastic changes in control sections. Exposure of this node to phosphate-water caused marked swelling of the cytoplasm of the lymphocytes. The reactivity of the nodes to hypotonic solutions may depend on the physiologic activity of the tissue in the animal. The number of lymph nodes studied, however, was not sufficient to establish definitely whether inflammatory processes in the node increase the reactivity of the tissues to hypotonic solutions.

Testis

The earliest effect of phosphate-water on the testis of a rabbit was observed when the tissue was incubated at 38°C. for ½ hour in a fluid consisting of 3 parts of phosphate-water and 7 parts of phosphate-Ringer's solution. The sections of the tissue showed an almost complete disappearance of mitotic figures. The nuclei of most of the spermatogenic cells were large and showed a few large chromatin granules.

Incubation with a more hypotonic solution (9 parts of phosphate-water and 1 part of phosphate-Ringer's) caused the testicular cells to become swollen until they completely filled the seminiferous tubules (Fig. 14). The cytoplasm was vacuolated and the nuclei stained faintly but maintained their structural characteristic of coarse chromatin granules. A few nuclei were very large and consisted of fine, lightly stained, basophilic granules. These giant nuclei did not have definite nuclear membranes. In contrast to the spermatogenic cells, the interstitial cells were well preserved, appeared normal in size, and had deeply staining nuclei and cytoplasm. In many experiments, however, no interstitial cells were seen in the sections; they may have been washed out from the tissue by the distilled water.

Exposure of rabbit testis to phosphate-water at 38°C. for 30 minutes caused almost complete destruction of the spermatogenic cells (Fig. 15). The tubules were filled with amorphous, eosinophilic débris. Some of the tubules, however, still had many well preserved, darkly staining, elongated nuclei of spermatocytes.

When the testis of the rat was exposed to hypotonic solutions, the spermatogenic cells were also destroyed with the production of eosinophilic detritus. There was a tendency, however, for the phosphate-water to remove or dissolve the débris, leaving the fibrous framework which usually became distorted and collapsed. The most satisfactory histologic picture was obtained in a rat testis incubated without shaking at 4°C. for 6 hours. The section consisted of thin fibrous strands which enclosed large, irregular-shaped, clear spaces (Fig. 16). In the persisting fibrous trabeculae there were numerous small blood vessels. The endothelial cells were enlarged and had clear cytoplasm and large, round, pale nuclei (Fig. 17). Many of the small arterioles had no endothelial cells, evidently as a result of their lysis by the phosphate-water. Capillaries were not seen in this section, but in testicular tissues exposed for a shorter time the capillaries were found to consist of swollen endothelial cells.

Testis exposed to buffered Ringer's solution at 38°C. for 1 hour showed a separation of the tubules, presumably due to intertubular edema (Fig. 18). Mitotic figures were numerous, although somewhat reduced in number as compared to the control, untreated tissue. The architectural arrangement of the cells in the tubules was well preserved. The cells and their nuclei were normal in size and in stainability.

Testis exposed to phosphate-sucrose solution at 2°C. for 3 hours showed, on microscopic section, a marked reduction in the number of mitotic figures. Many tubules had large giant cells with two to ten nuclei of moderate size, which were either vesicular or stained deeply and uniformly (Fig. 19). An occasional cell had a deeply staining, giant-sized nucleus. Multinucleated cells were also observed in testis exposed for a short time to a hypotonic solution and then to a phosphate-Ringer's solution.

Salivary Gland and Other Tissues

The submaxillary gland of the rabbit was exposed to phosphate-water at 38°C. for 1 hour. The sections had normal architecture (Fig. 21). There was no enlargement of the epithelial cells, not even of the peripherally placed ones which were in direct contact with the hypotonic solution. In contrast, the numerous arterioles throughout the tissue showed lysis of red blood cells and swelling of the endothelial cells. The nuclei of the cells of the tubules and acini were small, some-

what irregular in shape, and stained deeply and uniformly. These nuclei were apparently pyknotic. The cytoplasm of the tubules stained more deeply than in control sections. The observed changes in the nuclei and cytoplasm probably indicate that the epithelial cells were dead at the time of fixation.

The cells of the glandular tissue exposed to phosphate-Ringer's and sucrose solutions had, for the most part, nuclei which were fairly normal in microscopic appearance. A few nuclei, however, were small and stained deeply and homogeneously.

In sections of liver exposed to distilled water at 2°C. for 7 hours the peripheral zone consisted of enlarged cells with vacuolated cytoplasm and normal or small, darkly staining nuclei. The enlargement of the peripheral hepatic cells obliterated the sinuses. In the central part of the section, the architecture and the cellular detail were normal. In other experiments (liver exposed to 38°C. for 1 hour), the sinuses throughout the section were filled with deeply staining, homogeneous eosinophilic material.

Transplantable Tumors

A strain of leukemic myeloblastoma was grown as subcutaneous nodules in dba mice. The tumor was excised and exposed to phosphate-water at 38°C. for 30 minutes. Sections of the treated tissue (Fig. 22) showed a peripheral zone composed of eosinophilic débris and a few degenerating cells. Beneath the layer of detritus was a thin zone in which the cells were enlarged, with clear or lightly stained cytoplasm and large, muddy, lightly stained nuclei. In the center of the section the cells were well preserved and the nuclei well stained. Mitotic figures, however, were few in number although they were numerous in control sections.

Other transplantable tumors, sarcoma 180 and melanoma of mice, and R39 of rats, were exposed to phosphate-water at 38°C. for 1 hour and their behavior was found to be similar. Sections showed enlargement of the malignant cells, the cytoplasm was unstained, and the nuclei of most of the cells were apparently normal. A few nuclei near the periphery of the section were slightly enlarged and had a muddy stain. There were no mitotic figures.

Discussion

A Histologic Method of Studying the Physiology of Excised Tissues

The purpose of the customary histologic and pathologic methods is the determination of the morphologic appearance of the cells and tissues in the normal, diseased, or experimental animal. To accomplish this objective, the tissues are fixed, *i.e.*, killed, immediately after their

removal from the animal's body. The present series of experiments on cellular physiology has as its objective the determination of the reactions of living cells *in vitro* to reagents. It is well known that freshly excised tissues are composed of living cells. Any histologic changes occurring in the tissue after excision are the result of the reaction of the living, dying, or dead cells to the particular environment. This line of reasoning led to the utilization of the deferred histologic method in which the excised tissue is first exposed to the reagent, then fixed, and studied histologically.

A review of the literature shows that several investigators have used the deferred histologic method to study specific problems. Workers who use Warburg's manometric method [2] frequently examine tissue histologically after studying its metabolism. Usually these histologic studies are performed as a check on the type and morphologic integrity of the tissue. Sometimes the tissues were studied to determine the changes induced by the artificial environment. Okamoto,[3] for example, found that rat tumors subjected to anaerobiosis maintained their morphologic structure for 3 days at 37°C., whereas hepatic tissue under identical conditions developed degenerative changes. The deferred histologic method was used also by Colwell [4] who studied autolysis in excised hepatic tissue subjected to irradiation.

The deferred histologic method has a few limitations. In the first place, the method does not differentiate between living and dead cells. It should be emphasized that marked histologic changes do not necessarily mean that the cell is dead. On the other hand, certain chemicals (fixatives, for example) may kill the cell without producing any perceptible histologic change. A second limitation of the method is that the reagent is not in direct contact with all cells. The reactions, then, depend in part on the diffusibility of the reagent through the tissue. In many of the present experiments only the peripheral zone was affected. It is therefore important not to trim the tissue used for histologic section. A third limitation is the difficulty in obtaining quantitative data with this method inasmuch as histologic observations are usually not suitable for quantitative analysis. In spite of these limitations, the deferred histologic method has a distinct place in the armamentarium of the cellular physiologist.

The Effect of Hypotonic Solutions on Cells and Tissues

Cells differed markedly in their reaction to hypotonic solutions. At least three types of reactions were observed. One type was shown by the spermatogenic cells of the testis, the reticular cells of the thymus, and the endothelial cells of the blood vessels. On incubation

with phosphate-water, these cells became swollen, the cytoplasm stained lightly or was unstained, and the nuclei were large and poorly stained. Ultimately these cells were lysed, leaving a clear space or a small amount of eosinophilic débris.

The cells of the salivary gland exemplified a second type of reaction to hypotonic solutions. Exposure to phosphate-water did not enlarge the parenchymal cells or their nuclei and did not decrease the staining intensity of the cells. In fact, the nuclei stained more intensely after incubation of the living tissue with distilled water. This second type of reaction was observed in some experiments with cardiac muscle, the smooth muscle cells of blood vessels, and the stratified epithelium of the tongue.

A third type of reaction to hypotonic solutions was characterized by swelling of the cell, but the nucleus failed to enlarge. The cytoplasm stained poorly while the staining intensity of the nucleus was normal or greater than normal. Liver cells in contact with phosphate-water showed this type of reaction.

Some cells varied in their reaction to hypotonic solutions. In some experiments, the lymphocyte of the lymph node showed little or no histologic change. In other experiments, apparently under the same conditions, the lymphocyte became enlarged, the cytoplasm was voluminous and unstained, but the nucleus remained normal in size and staining intensity. A detailed review of the experimental and control sections was made to determine the reason for the variation in the reactions of the lymphocyte. It seemed that the resting lymphocyte of a normal lymph node did not become enlarged on treatment with distilled water but the active lymphocyte of inflammatory nodes apparently reacted strongly to the hypotonic solutions. These findings suggested that the reactivity of the lymphocyte to distilled water depended on the physiologic state of the cell.

The histologic changes that were observed in cells treated by hypotonic solutions are evidently the reflection of physiologic reactions within the cell. The enlargement of the spermatogenic, reticular, and endothelial cells in hypotonic solutions and the poor staining of the cytoplasm are evidently the result of the excessive penetration of water into the cell. The enlargement and the light staining of the nuclei of these cells may be assumed to be due to the entrance of water into the nuclei. Evidently, the cell wall and the nuclear membrane of these cells are permeable to water. On the other hand, certain cells, such as the parenchymal cells of the salivary gland, failed to enlarge when treated with phosphate water. This finding may be interpreted in two ways: Either the cell wall is not readily permeable to water or it is

so rigid that it does not permit the enlargement of the cell. The enlargement of some types of cells (hepatic cells and lymphocytes of inflammatory lymph nodes) without an associated enlargement of the nuclei may be interpreted as indicative of permeability of the cell wall to water but an impermeability or a rigidity of the nuclear membrane. It would seem, then, that the observed differences in the reactions of cells to hypotonic solutions are probably due to differences in the characteristics of the cell wall and the nuclear membrane.

The histologic changes within the cells treated with hypotonic solutions were associated with the formation of extracellular precipitates. Different types of precipitates were observed. In two isolated experiments a small amount of precipitate that stained homogeneously with eosin developed in the medulla of a lymph node and in the sinuses of hepatic tissue treated with phosphate-water. The precipitates were not definitely associated with the lysis of cells and they may have resulted from the exudation of a protein from the intact cells.

A heavy, amorphous, eosinophilic precipitate was formed as a result of the lysis of the spermatogenic cells of the testis of the rabbit. In contrast, no definite precipitate followed the lysis of the reticular cells of the thymus and the endothelial cells of the blood vessels. It seems that the products released by the lysis of the reticular and the endothelial cells were washed away or dissolved in the supernatant fluid, but the products of lysis of the testicular cells of the rabbit were less soluble.

In some of the experiments with lymph node or spleen there was formed a granular or fibrillar precipitate which was eosinophilic or slightly basophilic. The precipitate was presumably the result of the lysis or the rupture of lymphocytes. The inconstancy of the formation of the precipitate in different experiments may be due to a tendency of the supernatant fluid to remove the precipitate from the tissue.

Exposure of thymic tissue to hypotonic solutions resulted in the formation of a characteristic precipitate which was usually fibrillar in structure and was basophilic in staining. The formation of this precipitate in thymic tissue is evidently correlated with the gelatinous precipitate which was previously observed on the addition of distilled water to suspensions of thymic cells.[1] The histologic sections indicated that the precipitate was derived from the small cortical cells of the thymus. It is possible that Hassall's corpuscles played a rôle in the formation of the basophilic, fibrillar precipitate.

Summary

Freshly excised tissue was exposed to distilled water at 2°, 38°, and 45°C. and was then fixed, sectioned, and stained.

Under these conditions the testis showed disappearance of mitotic figures, then swelling and ultimately lysis of the spermatogenic cells. The fibrous framework, however, was well preserved.

In the thymus, the reticular cells underwent swelling and lysis. The cortical cells developed nuclear changes and were finally replaced by a fibrillar or granular, basophilic precipitate.

The reaction of the spleen and lymph node to hypotonic solutions varied and was usually slight. In some cases, however, the lymphocytes became enlarged and occasionally a small amount of granular or fibrillar precipitate developed.

Exposure to distilled water did not affect the acini or ducts of the salivary gland but did cause pyknosis of the nuclei.

Blood vessels showed enlargement and finally lysis of the endothelial cells and little or no change was seen in the muscularis.

The cells of a few transplantable tumors became large and edematous and, in some cases, showed degenerative changes.

Incubation of the testis with 0.33 molar sucrose solution resulted in the formation of large multinucleated giant cells in the tubules.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 193

FIGS. 1, 2, and 3. Thymus (rat) exposed to phosphate-water (5 per cent phosphate buffer, pH 7.6, in distilled water) for 30 minutes at 2°C. × 700.

FIG. 1. The small cortical cells of the thymus are separated from each other by a small amount of intercellular edema. The cells are normal in size or slightly enlarged. The nuclei stain deeply and have the shape of a horseshoe or ring.

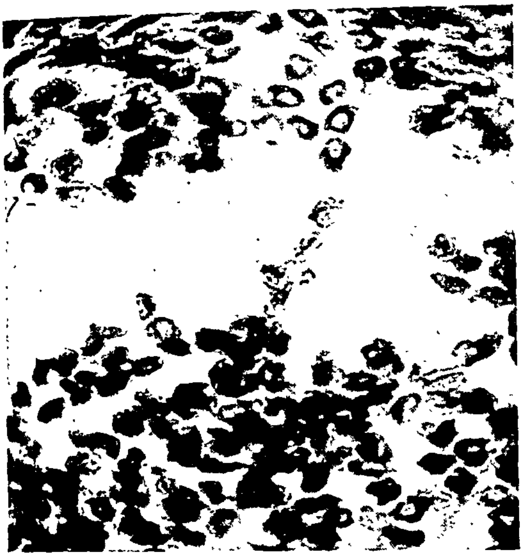
FIG. 2. The photomicrograph is taken from an area composed of a mixture of cortical and reticular cells. The cortical cells are small and have small, deeply staining nuclei. The reticular cells are enlarged, the cytoplasm is unstained, and the nuclei are considerably enlarged and stain lightly. A few nuclei have prominent nucleoli.

FIG. 3. The small acinus-like structure is composed of two flattened cells with deeply staining cytoplasm and nuclei. The cells surround a small, clear space. A few basophilic fibrils loop around the acinus-like structure.

FIGS. 4 and 5. Thymus (rat) exposed to phosphate-water for 3 hours at 2°C. × 350.

FIG. 4. The cortical cells are normal in size and have small, dark, nuclei. The reticular cells are markedly enlarged and have unstained cytoplasm; the nuclei stain very faintly. The nuclei of some reticular cells are not seen in this section.

FIG. 5. The cortical cells have been largely replaced by several bundles of fine and coarse, deeply staining fibrils which loop around a poorly preserved acinus-like structure. The reticular cells in the medulla of the thymus have completely disappeared leaving large, clear spaces.



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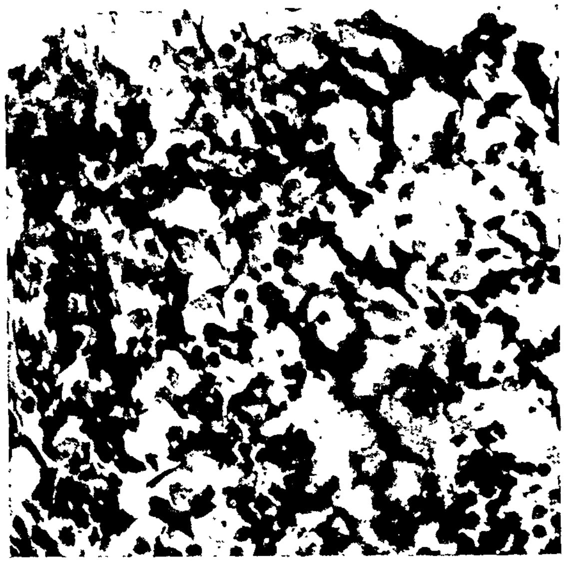

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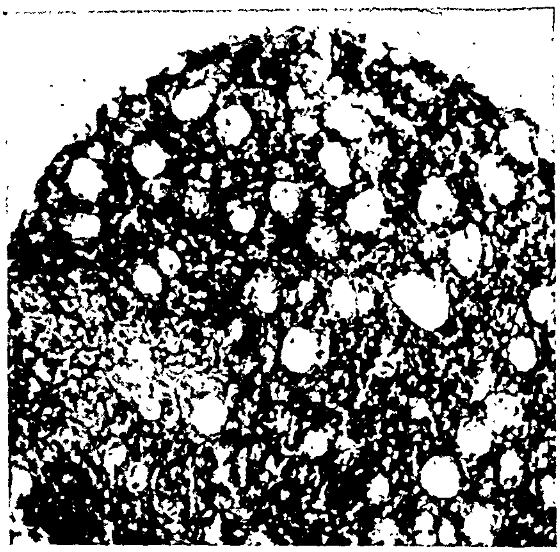

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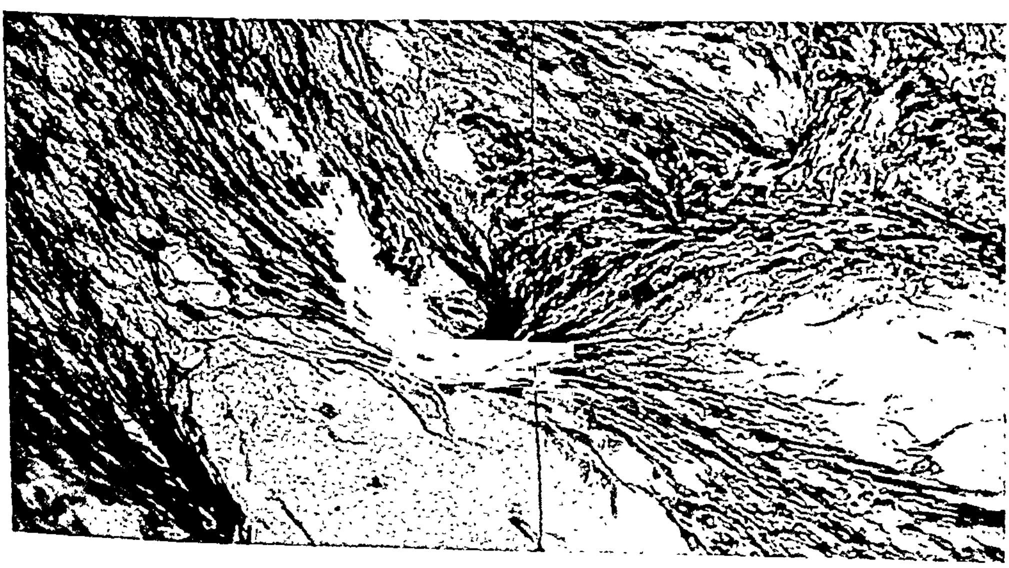


Schrek

5

Reactions to Hypotonic Solutions

PLATE 194

FIG. 6. Thymus (rabbit) exposed for 30 minutes at 38°C. to a mixture of 7 parts of phosphate-water and 3 parts of phosphate-Ringer's solution. × 170. The cells in the peripheral zone are separated from each other to a slight extent by intercellular edema. The peripheral cortical cells are normal in size and have small, round nuclei which stain less intensely than normally. The cells below this peripheral zone are normal in size and staining intensity.

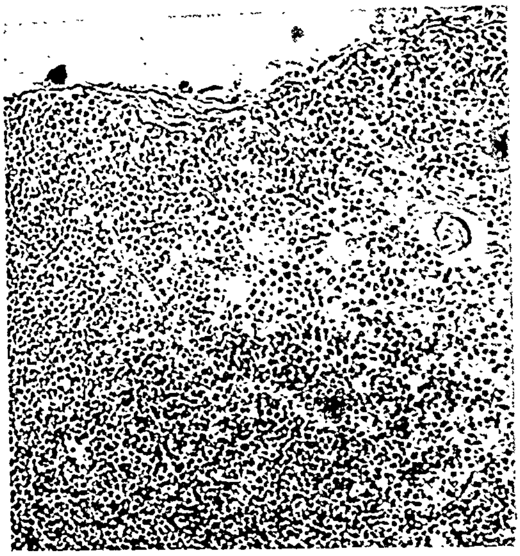
FIG. 7. Thymus (rabbit) exposed to phosphate-water for 1 hour at 38°C. × 350. The tissue has a granular and fibrillar precipitate which is basophilic in the stained section. The persisting cortical cells have slightly enlarged, deeply staining nuclei.

FIG. 8. Thymus (rabbit) exposed for 30 minutes at 38°C. to a mixture of 9 parts of phosphate-water and 1 part of phosphate-Ringer's solution. × 700. The figure shows a longitudinal section of a large blood vessel. The endothelial cells are enlarged with large, spindle-shaped nuclei. Beneath the endothelium is a layer of enlarged cells with clear, unstained cytoplasm. The round nuclei of the cells stain slightly. These cells presumably are smooth muscle cells of the muscularis. The outer layer of the blood vessel is composed of collagenous fibers.

FIG. 9. Thymus (rabbit) exposed to phosphate-Ringer's solution for 4 hours at 45°C. × 350. The cortical cells are sparse in number. The persisting cells are normal in size and have round, dark nuclei. The blood vessel in the upper part of the section has many fairly well preserved red blood cells and a few leukocytes.

FIG. 10. Spleen (rat) exposed to phosphate-water for 6 hours at 2°C. × 350. The tissue has a thick, fibrillar precipitate which is eosinophilic in the stained section. In the interstices of the fibrils are a few enlarged cells with clear cytoplasm and pale nuclei.

FIG. 11. A human, cervical lymph node treated with phosphate-water for 30 minutes at 38°C. × 350. The lymphocytes are markedly enlarged with clear, unstained cytoplasm. The cell walls are prominent. Some nuclei are normal in size and dark in color and others are enlarged and stain lightly.



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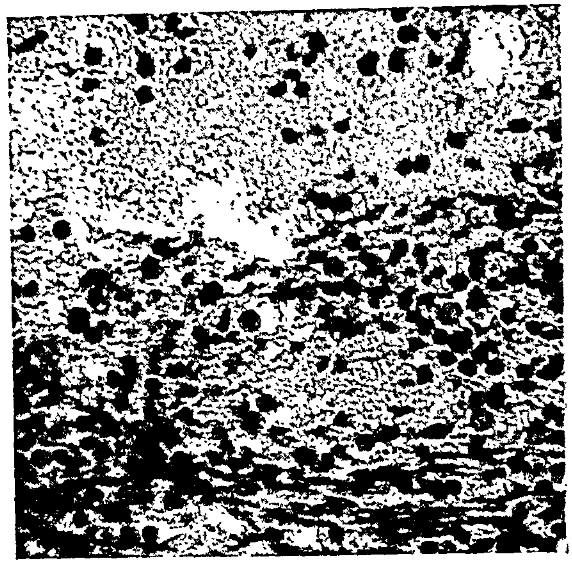

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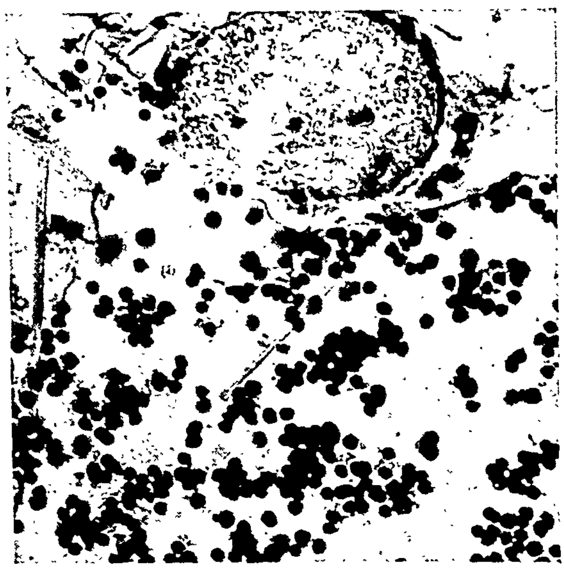

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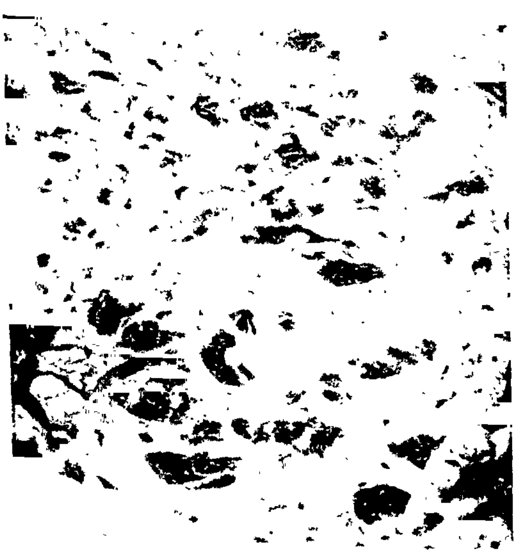

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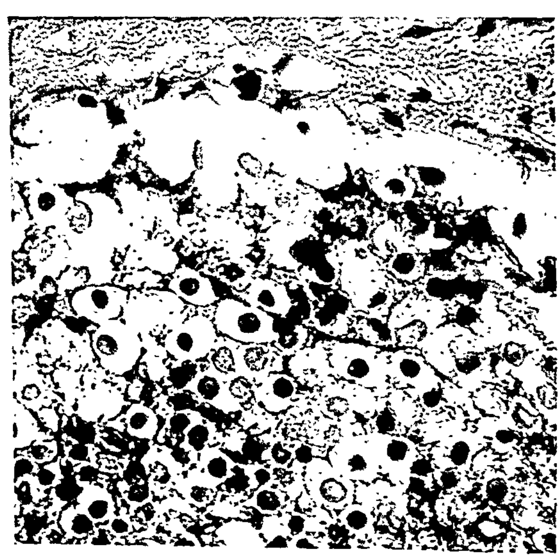

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Reactions to Hypotonic Solutions

PLATE 195

FIG. 12. A cervical lymph node of a rabbit. The node was exposed to distilled water for 5 hours at 2°C. × 350. In one area the cells are replaced by a fibrillar and granular precipitate. The persisting lymphocytes are enlarged. The cytoplasm of the cells is increased in volume and is unstained. The nuclei are normal in size and stain deeply.

FIG. 13. A para-aortic lymph node of a rabbit. The tissue was subjected to distilled water for 30 minutes at 38°C. × 350. The lymph follicle is composed of lymphocytes which are normal in size and in staining intensity. The medulla has few cells, both in this section and in control sections of untreated tissue.

FIG. 14. Testis (rabbit) incubated for 30 minutes at 38°C. with a mixture of 9 parts of phosphate-water and 1 part of phosphate-Ringer's solution. × 350. The spermatogenic cells are enlarged, filling the tubule. The cytoplasm is unstained. The nuclei are very large and stain poorly. No mitotic figures are present.

FIG. 15. Testis (rabbit) incubated with phosphate-water at 38°C. for 30 minutes. × 350. The tubule is filled with amorphous eosinophilic débris. No spermatogenic cells can be seen. Many small, spindle-shaped, deeply stained nuclei of spermatocytes are still present in the tubule.

FIGS. 16 and 17. Testis (rat) treated with phosphate-water for 6 hours at 4°C.

FIG. 16. The fibrous framework of the tubules is well preserved. A few blood vessels are present in the fibrous tissue. The spermatogenic cells and the spermatozoa have disappeared, leaving empty spaces in the tubules. A small amount of amorphous detritus persists in a few tubules. × 120.

FIG. 17. The endothelial cells of the small arteriole are cuboidal and markedly enlarged. The cell walls are prominent and the cytoplasm is unstained. The nuclei are large, round, and lightly stained. The smooth muscle cells in the muscularis are not enlarged and have deeply staining cytoplasm and nuclei. There is some enlargement of the nuclei of these cells. × 700.

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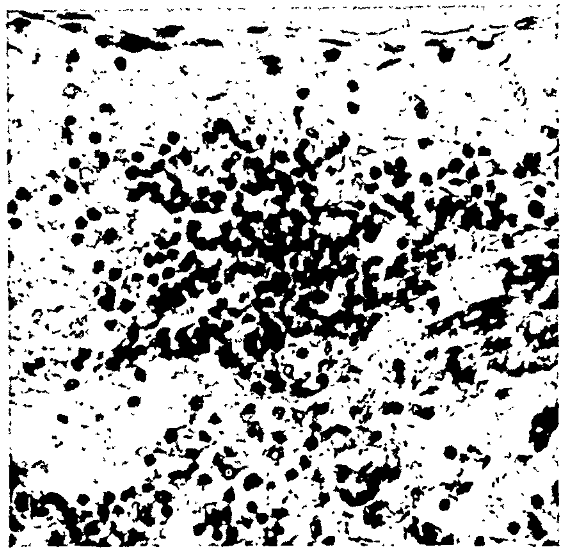

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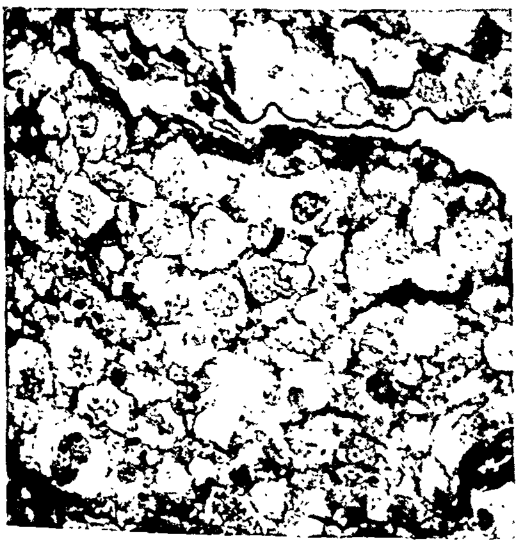

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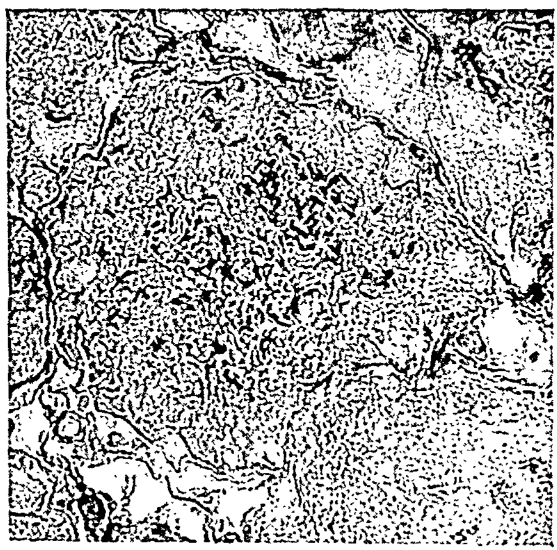

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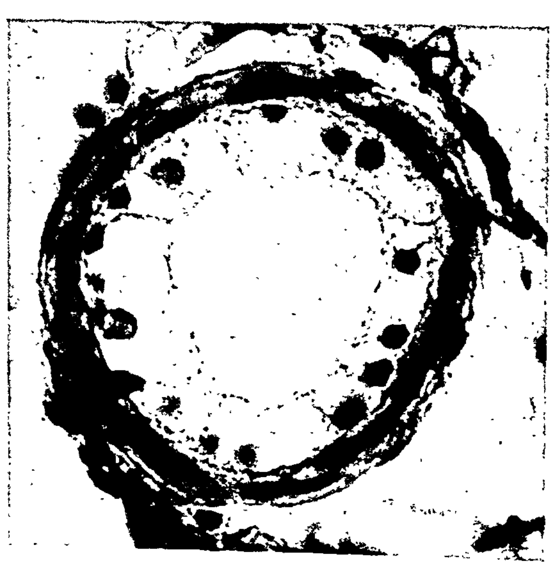

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Reactions to Hypotonic Solutions

PLATE 196

FIG. 18. Testis (rabbit) incubated with phosphate-Ringer's solution for 1 hour at 38°C. × 170. The cells in the tubules have separated from the basement membrane, probably due to intertubular edema. The cellular architecture of the tubules is well preserved. The cells are apparently normal in size, and the cytoplasm and nuclei stain deeply. Many cells are in mitotic division.

FIG. 19. Testis (rabbit) exposed to phosphate-sucrose solution (5 per cent phosphate buffer, pH 7.6, in a 0.33 molar solution of sucrose) for 3 hours at 2°C. × 250. The tubule has numerous, large, multinucleated cells with deeply staining cytoplasm. The cells have 2 to 10 fairly large nuclei which are vesicular in some cells and which stain deeply and uniformly in other cells.

FIG. 20. A normal salivary gland of a rabbit. × 350. The tissue was fixed immediately after excision. The blood vessel beneath the duct is filled with red blood cells.

FIG. 21. Salivary gland of a rabbit. The tissue was incubated with phosphate-water for 1 hour at 38°C. × 350. The cells in the tubule are not enlarged. The cytoplasm stains intensely. The nuclei are normal in size and stain deeply and uniformly. A small arteriole below the duct has enlarged endothelial cells and does not contain any blood cells. The periductal fibrous tissue is edematous. The acinar cells are normal in size. The cytoplasm is granular as in the control section. The nuclei are somewhat smaller than normal, slightly irregular, and stain deeply.

FIG. 22. Transplantable leukemic myeloblastoma grown as a subcutaneous nodule in a mouse. The tumor was incubated with phosphate-water for 30 minutes at 38°C. × 350. The section has three distinct zones. The peripheral zone at the right is composed of amorphous, eosinophilic débris with an occasional, persisting nucleus. These features result from the experimental procedure. A second layer is composed of enlarged cells with clear cytoplasm and enlarged, lightly staining nuclei. The third layer is composed of leukemic cells which are normal in appearance.

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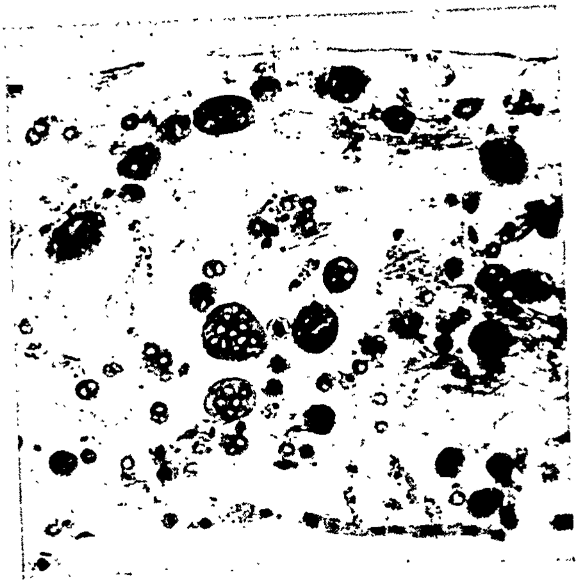

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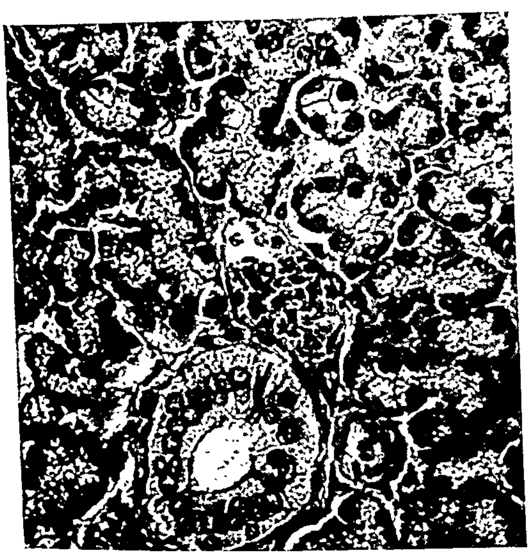

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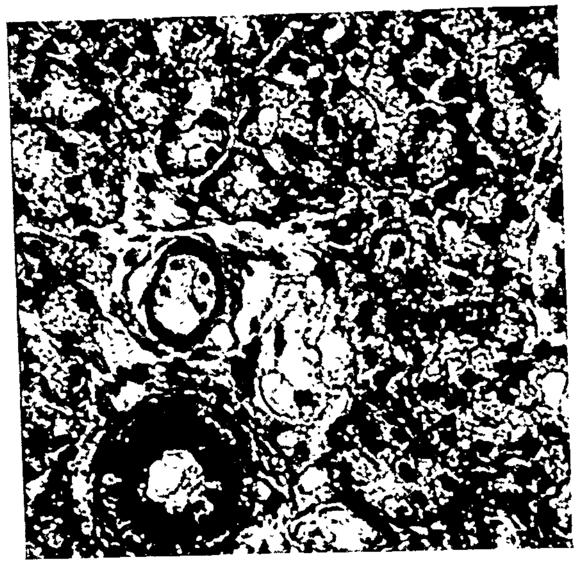

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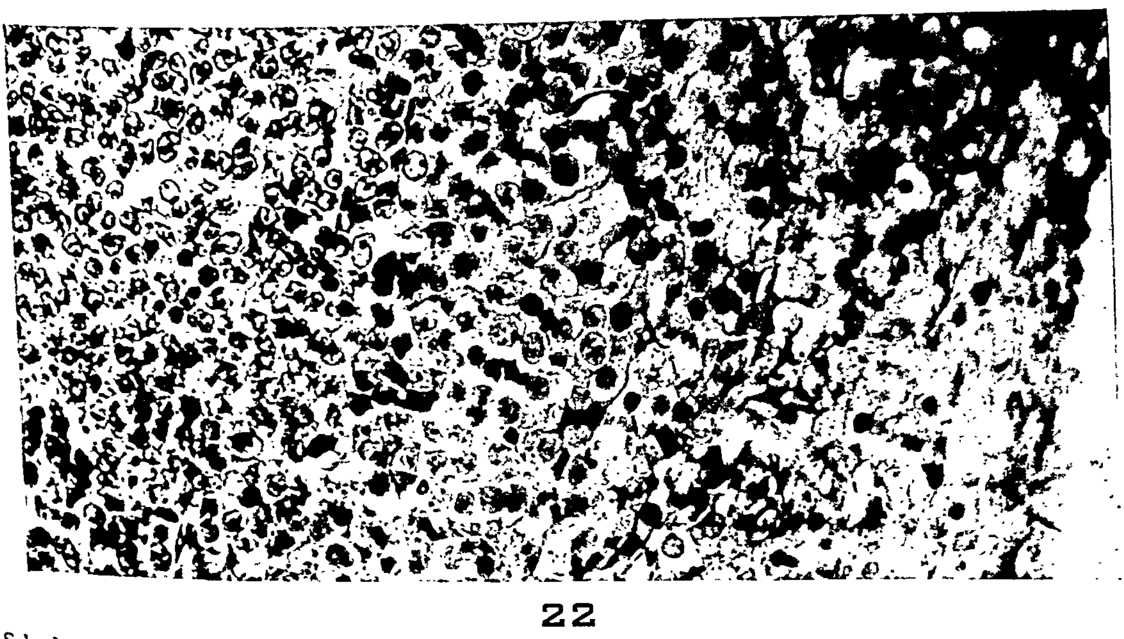

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A PATHOLOGICAL STUDY OF MICE INFECTED WITH THE VIRUS OF SWINE INFLUENZA *

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Since the demonstration by Andrewes, Laidlaw, and Smith[1] of the susceptibility of mice to the viruses of human and swine influenza, white mice have been used extensively in the study of these diseases. Several detailed reports[2-7] of the lesions produced in mice by infection with the virus of human influenza have been presented. On the other hand I have been able to find only one paper dealing with the detailed histopathology of the infection in mice by the virus of swine influenza. In that paper Straub[5] described the lesions produced in the respiratory tract by the viruses of both human and swine influenza. The changes were apparently identical and were described together. Both viruses affected specifically the epithelium of the respiratory tract from the bifurcation of the trachea to the bronchioles, producing a necrosis followed by proliferation and regeneration of the epithelium. Catarrhal bronchitis was followed by collapse of the lung tissue. Straub stated that the pulmonary changes were secondary to the bronchitis and that there was no real pneumonia. He described only the bronchi and lungs and made no mention of lesions in other organs.

Because only the bronchi and lungs have heretofore been described, the present experiment was done in order to make complete anatomical studies of mice receiving intranasal inoculation of the virus of swine influenza.

Materials and Methods

White mice, 4 to 5 weeks old, were used. The virus of swine influenza, obtained from Dr. J. W. Beard, had been passed several times in chicken embryos and was received in the form of a bacteria-free suspension in chorioallantoic fluid.

After the mice were anesthetized with ether, 0.05 cc. of the virus suspension was introduced into the nose with a tuberculin syringe. There were two test groups. One group received the undiluted suspension of virus in chorioallantoic fluid. Of this group, 74 were examined microscopically after a period of infection ranging from 6 hours to 35 days. The potency of this suspension was such that the 50 per cent end point[8] for gross pulmonary lesions was a dilution of $10^{-7.7}$. In the other group the inoculum consisted of a 10 per cent suspension

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in broth of lungs from mice previously inoculated with the virus. Cultures of this suspension showed a light growth of *Bacillus coli* and nonhemolytic streptococci. As will be seen later, these bacteria were of no significance in the development of the lesions. Of this second group, 73 mice were examined microscopically at intervals ranging from 6 hours to 28 days. The 50 per cent end point for gross pulmonary lesions of this inoculum was a dilution of $10^{-5.4}$.

Controls consisted of 23 untreated mice, 19 mice subjected only to ether anesthesia, 26 mice receiving intranasal inoculation of normal mouse lung, and 20 mice receiving intranasal inoculation of normal chorioallantoic fluid. These animals were killed at intervals ranging from a few hours to 28 days.

Because small numbers of bacteria were present in the virus-lung inoculum and because similar bacteria were later cultured from the lungs of some of the test animals, a further control experiment was done in order to determine whether intranasal inoculation of the bacteria alone could produce pneumonia in mice. A Salmonella and a strain of *B. coli* were isolated from the lungs of mice which had been inoculated with the virus of swine influenza. Forty-eight hour cultures of the bacteria grown on Douglas agar slants were suspended in physiological saline solution so that 1 cc. of the suspension contained approximately one-half billion organisms as determined by turbidity standards. In addition, a 1:200 dilution of each of the two suspensions (Salmonella and *B. coli*) was made. Of these four bacterial suspensions, 0.05 cc. was inoculated intranasally after ether anesthesia, using four groups of mice. One group of 8 mice received approximately 25 million Salmonella organisms. Another group of 7 mice received about 125,000 Salmonella organisms. Two other groups of 7 mice each received like numbers of *B. coli* organisms respectively. A fifth group of 5 mice was given sterile saline washings of Douglas agar slants. All mice in these five groups survived the inoculation and were killed on the fourth day. The lungs from about one-half of them were cultured.

As an additional check on the identity of the infecting agent in the test animals, protection tests were made with serum from swine vaccinated with formalin-inactivated virus of swine influenza. A mixture of immune swine serum and virus produced no lesions in mice, while a mixture of normal swine serum and virus produced gross pulmonary lesions.

Complete autopsies were performed shortly after death on animals that died. Other animals were killed with ether and examined immediately. In some cases the lungs were removed under sterile conditions and cultures made. The tissues were fixed in either Zenker-

acetic or Zenker-formalin solutions. After the skull was decalcified, sections were made of the nose and nasopharynx. Sections for microscopical study were made from paraffin blocks and were stained with hematoxylin and eosin and with MacCallum's bacterial stain.[9]

RESULTS

Clinical. Symptoms of infection appeared 1 to 3 days after inoculation of the virus. The mice became comparatively inactive and ate and drank less. The fur became ruffled. Respiratory movements were exaggerated and breathing was labored. Most deaths occurred between the 2nd and 5th days, the maximum number occurring on the 4th day. Animals died as early as 18 hours and as late as 9 days after inoculation of the virus. All mice that died had gross pulmonary lesions.

Bacteriological. In the group of mice which received the virus-egg suspension, cultures of the lung showed a very light growth of *B. coli* on the 1st day and were negative thereafter. No bacteria were seen in sections. The presence of both gram-positive and gram-negative bacilli in the intestine on the same slides as the lungs served as a control for the stain. In the group of mice which had received the virus-lung suspension, cultures of the lungs showed a very light growth of *B. coli* and α-streptococci in the first 48 hours and were negative thereafter. No bacteria, however, were seen in sections.

Cultures of the lungs from the mice which received the bacterial suspensions were negative in about half the cases and in the other half yielded a light growth of the inoculated organism. No growth was obtained from cultures made of the lungs of mice inoculated with the washings of agar slants. No bacteria were seen in the specially stained sections of lungs of any of these groups. Apparently too few of these gram-negative organisms remained in the lung on the 4th day to be discoverable in the sections.

Cultures of lungs from the remaining control groups were all negative for bacteria; no bacteria were seen in sections.

PATHOLOGICAL ANATOMY OF THE TWO GROUPS RECEIVING THE VIRUS

Gross Lesions

Except for rare and minimal changes in other organs, all important lesions were confined to the lower part of the trachea, the bronchi, and the lungs. The anatomical changes were, with the exception of difference in severity of alveolar necrosis, identical in both groups of mice, and will be described together.

All mice that died had gross pulmonary lesions. The mouse that died in 18 hours showed consolidation of approximately 10 per cent of

the pulmonary tissue. All mice that died subsequently had gross involvement of at least 70 per cent of the pulmonary tissue. The solidified areas were of a bluish purple, homogeneous appearance. The lungs were relatively airless and sank in the fixing fluid. When smaller areas of the lungs were involved, these tended to be localized to the hilus and posteriorly. In the first few days the affected regions were quite wet but later became dry.

Microscopical Lesions

At 6 Hours. Lesions were found in 3 of 7 animals. The lower portion of the trachea contained a few polymorphonuclear leukocytes; the tracheal epithelium did not appear abnormal. The larger bronchi were filled with pus, and many polymorphonuclear leukocytes were present in all coats. The bronchial epithelium showed no evidence of necrosis at this time. The pulmonary lesions were very slight and restricted to small areas around the bronchi. These areas showed interstitial infiltration by many polymorphonuclear leukocytes and a small number of mononuclear cells. The alveoli were empty. One outstanding feature was a marked edema of the lymphatics around the larger bronchi and vessels.

At 18 to 24 Hours. Lesions were present in 6 of 7 animals. One died at 18 hours. The tracheal epithelium showed slight focal necrosis and was covered by a coat of polymorphonuclear leukocytes. The submucosa also contained leukocytes. Pus was present in the bronchi, bronchioles, and regional alveolar ducts. There was increased secretion of mucus by the bronchial epithelium. There was now definite evidence of necrosis and desquamation of the bronchial and bronchiolar epithelium (Fig. 2). Necrosis of the cells lining the alveoli was present in a few foci. The walls of the bronchi and bronchioles contained many polymorphonuclear leukocytes. Occasionally a bronchus was so plugged with pus that collapse of the corresponding portion of the lung resulted; this, however, was not a prominent feature at this stage. The lesion was still mainly bronchial. The alveoli for the most part were empty and the interstitial reaction was slight; polymorphonuclear leukocytes predominated although mononuclear cells also were present. The dilatation of the peribronchial and perivascular spaces was quite marked. An occasional arteriole and vein showed penetration of all coats by small numbers of polymorphonuclear leukocytes.

At 2 Days. Changes in the trachea reached a maximum 2 days after infection, but were only slight compared to those of the bronchi and alveoli. In a few areas the tracheal epithelium consisted of a single

layer of flat cells. Elsewhere, hyperplasia of the tracheal epithelium was in progress (Fig. 9).

Necrosis of the bronchial and bronchiolar epithelium was considerable, and that of the lining cells of the alveoli moderate. In some areas the epithelium of the bronchi and bronchioles was completely denuded; in others there was a single layer of flat epithelium. The necrotic lining often appeared as a pink hyaline membrane. Many small bronchi were now stuffed with pus and atelectasis was prominent. The interstitial exudate was now more diffuse and contained more cells, with polymorphonuclear cells predominating. The polymorphonuclear leukocytes were at their maximum number on this day. Mononuclear cells began to appear in moderate numbers in the bronchial wall, the lymphatics, and the interstitial exudate. The alveoli contained fluid, mainly, and small numbers of polymorphonuclear leukocytes, mononuclear cells, desquamated alveolar lining cells, and débris; there was no fibrin. In the mice that died there was marked congestion and some hemorrhage as well as a considerable degree of edema. The lymphatics were still prominent and contained more cells (Fig. 1).

At 3 Days. Three days after infection the necrotic process in the tracheal epithelium had just about disappeared, while a slight degree of hyperplasia persisted. Necrosis of the lining of the bronchi and alveoli had almost reached its peak. Numerous examples of a hyaline membrane were seen in bronchioles, alveolar ducts, and alveoli proper. Many bronchi and bronchioles were stripped clean of epithelium, while others showed a single layer of flat cells (Fig. 8). The interstitial cells were more prominent now, with polymorphonuclear and mononuclear cells about equal in number. There was more congestion, focal hemorrhage, and edema than on the 2nd day. In the mice that died spontaneously the interstitial cellular reaction was less, while congestion, edema, and hyaline necrosis of the epithelium were all more prominent than in the mice that were killed. The bronchial epithelium was beginning to show signs of regeneration in the form of a single layer of large polymorphic cells.

At 4 Days. The necrotic process of the bronchial epithelium was at its worst 4 days after infection and that of the alveolar cells followed closely. The necrotic lining was often seen as a cast which had fallen away from the wall. Proliferation of the epithelium of the larger bronchi was present to a moderate degree, producing two or three layers of cells (Fig. 10). These cells had large, dark nuclei with prominent nucleoli; some mitotic figures were seen. The arrangement of the nuclear material produced no uniform pattern. No inclusion

bodies were identified. A lesser degree of proliferation was seen in the smaller bronchi and bronchioles. In addition there was a slight proliferation of the cells lining the alveoli resulting in small clumps of three or four cells; this was present as much at the periphery of the lung as at the hilus and one received the impression that it was the result of multiplication of alveolar lining cells themselves rather than of downgrowth of cells from the bronchioles. The latter process was to become prominent later in the disease. The number of cells in the interstitial tissues had increased, with mononuclear cells about twice as numerous as polymorphonuclear cells. The animals that died showed more necrosis and less proliferation of the lining cells of the bronchi and alveoli than those that were sacrificed.

At 5 Days and Beyond. The proliferation of the tracheal epithelium, never more than slight, was absent by the 7th day.

The necrotic process in the bronchial epithelium was still marked on the 5th day but rapidly decreased thereafter, being absent entirely by the 7th day (Figs. 4 to 7). In the animals which received the virus-lung suspension, alveolar necrosis reached its maximum intensity on the 4th and 5th days and was gone by the 8th day. In the other group (virus-egg suspension), alveolar necrosis was less severe and proceeded more slowly, reaching a peak between the 6th and 8th days and disappearing entirely by the 10th day.

The proliferative process proceeded at different rates in the large bronchi, small bronchi, and alveoli. In the large bronchi hyperplasia was well marked on the 5th day, reached its maximum on the 6th and 7th days, and disappeared between the 11th and 17th days. In the smaller bronchi and bronchioles hyperplasia reached its maximum intensity between the 8th and 10th days and disappeared between the 14th and 20th days (Figs. 11 and 12). Many of the superficial cells of the proliferating epithelium underwent desquamation. When the proliferative process in the bronchial epithelium had disappeared, the cells appeared more or less normal, showing restoration of cilia and return of the nucleus to the base of the cell.

The proliferation in the bronchioles resulted in the formation of solid clumps of epithelial cells which at first remained confined to the bronchiolar lumen but soon extended into the alveoli (Figs. 13 and 14). The latter process was present to a moderate degree on the 7th day and reached its maximum between the 10th and 12th days. The peripheral portions of the lungs which had not as yet been invaded by this process still showed proliferation of the lining cells of the alveoli. Soon these two hyperplastic processes fused. The solid plugs of cells often underwent squamous metaplasia, and intercellular bridges

could be identified. It seemed that many of these occluded alveoli opened up again to some extent as a result of degeneration of the epithelial plugs. About the 14th day the nuclei of the cells forming the plugs became swollen and pale and the prominent dark clumps of chromatin were replaced by fine, pale granules (Fig. 15). In other plugs, the center contained hyaline masses of necrotic cells. Eventually the alveoli were lined by a single layer of cuboidal epithelium. The central hyaline masses were surrounded by polymorphonuclear leukocytes (Figs. 16 and 17). These leukocytes, which had almost disappeared by the 5th day, returned in small numbers but were restricted to the lumina of those alveoli which contained this hyaline débris. The process of degeneration of the solid plugs and partial reopening of the alveoli was well established by the end of the 3rd week. At this time the lung looked like a gland, the alveoli being lined by tall epithelial cells. This appearance was still present at 5 weeks, the end point of this experiment. Even though some alveoli were thus reopened, the affected portions of the lung were probably of little use in aeration of the blood since the alveolar lumina were small and the interstitial tissues considerably thickened as a result of infiltration by mononuclear cells. Moreover, many parts of the lungs were completely collapsed and solid.

In the meantime, the edema of both the alveolar lumina and the lymphatics was decreasing and was much less by the 10th day. Interstitial mononuclear infiltration was prominent on the 5th day and was at its height between the 6th and 8th days. Thereafter it decreased slowly and was still present in a slight to moderate degree at the end of the 5th week.

Differences Between Group Receiving Virus-Egg Suspension and Group Receiving Virus-Lung Suspension. Two differences were seen between the group of mice receiving the virus-egg suspension and the group receiving the virus-lung suspension. In the latter the necrosis of the alveolar lining cells was more extensive and occurred earlier in the disease. In addition, although the exact mortality rates were not established since some animals were killed that were obviously moribund, the mortality rate in the virus-lung group was about twice that in the other.

Changes in Other Organs. Changes other than in the lungs were rare. In the first few days after infection the mediastinal lymph nodes showed distention of the sinusoids with fluid as well as with many macrophages and a few polymorphonuclear leukocytes. In one mouse killed at 5 days there were several small perivascular collections of mononuclear cells in the myocardium. In one mouse which died at 5

days an artery near the epididymis showed infiltration by polymorphonuclear leukocytes. Slight leukocytic infiltration of one adrenal was present in one mouse killed on the 7th day. Two other findings of common occurrence were present in the test and control groups with equal frequency: Mucus and pus in the nose, and focal collections of mononuclear cells and leukocytes in the liver.

PATHOLOGICAL ANATOMY OF CONTROL GROUPS

Control Groups Receiving Bacterial Suspensions. Of 8 mice which received the undiluted suspension of Salmonella, 4 were normal and 4 showed a slight degree of interstitial mononuclear pneumonia. In the latter the bronchial epithelium was normal except for an increased secretion of mucus. The alveolar lumina were empty. Small numbers of mononuclear cells and fewer polymorphonuclear leukocytes were present in the interstitial tissues of the lung. No other abnormalities were seen. There was nothing comparable to the severe bronchitis and pneumonia produced by the virus. Of 7 mice which received the diluted suspension of Salmonella, only 2 showed a slight degree of interstitial reaction; the other 5 were normal. In the group of 7 mice which received the undiluted suspension of *B. coli,* 3 were normal and 4 showed an interstitial mononuclear response similar to that elicited by the Salmonella. Of 7 mice which received the diluted suspension of *B. coli,* only 1 showed any degree of interstitial reaction. Of the 5 mice which were inoculated with the agar washings, 3 were normal and 2 showed a minimal cellular infiltration of the peribronchial interstitial tissues.

Remaining Control Groups. The mice in the remaining control groups showed no lesions except for slight leukocytic infiltration in the interstitial tissues of the lung. In a few animals small collections of polymorphonuclear leukocytes were seen in the lumina of the bronchi in the first few days.

DISCUSSION

The lesion produced in mice by infection with the virus of swine influenza consisted essentially of necrosis of the epithelium of the bronchi and bronchioles, commonly producing hyaline membranes, together with a marked reparative proliferation of this epithelium resulting finally in restoration of the normal lining epithelium. In the bronchioles the proliferative process was so active that the hyperplastic epithelium grew into and filled the regional alveoli. The hyperplasia began shortly after the onset of necrosis and the two processes,

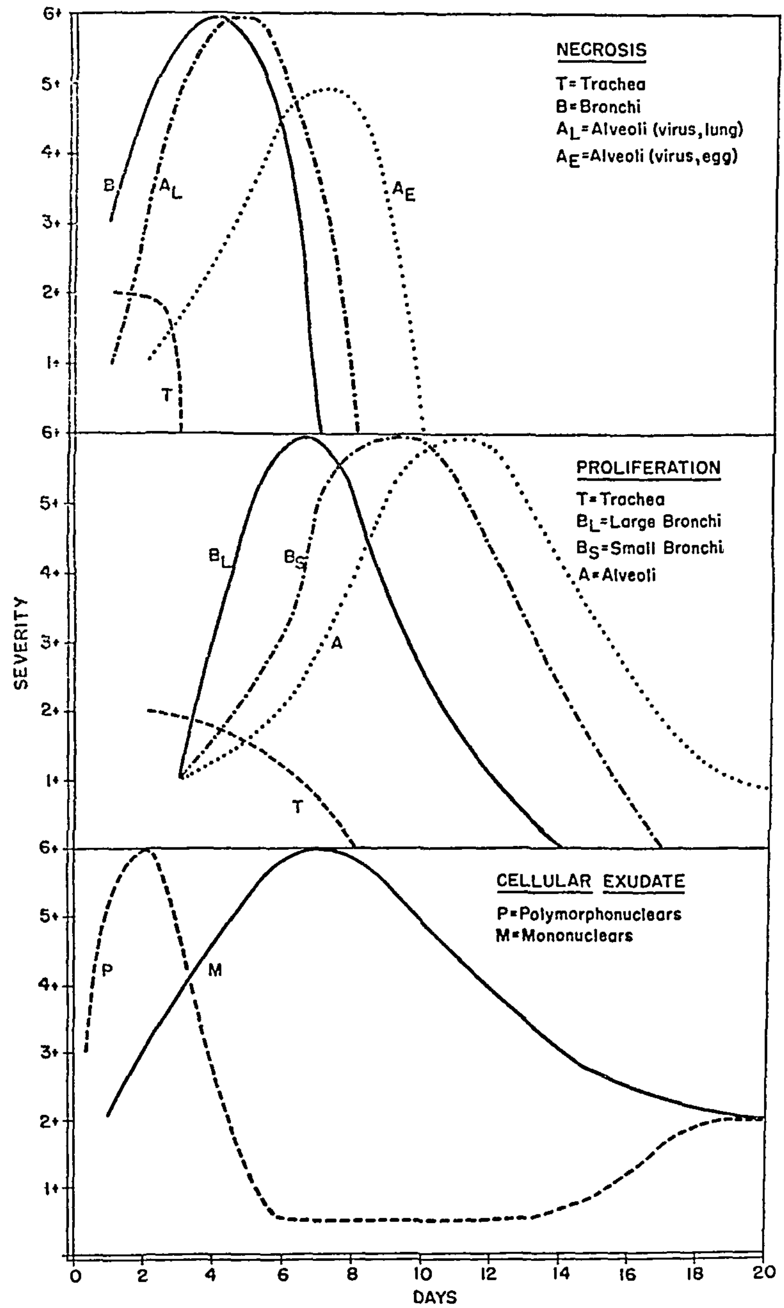
necrosis and proliferation, went on side by side. A similar lesion, although of much less intensity, occurred in the lower portion of the trachea. In addition to bronchitis there was also a pneumonia characterized by a hyaline necrosis of the alveolar lining and an interstitial exudate which was predominantly polymorphonuclear in the first 2 days and mononuclear thereafter. The pneumonia was further complicated by large areas of atelectasis which appeared as early as the 2nd day and resulted from obstruction of the bronchi by exudate, mucus, and desquamating epithelium. Deaths occurred mainly on the 4th day and were apparently caused by two main factors, asphyxia and viral intoxication. There were three factors involved in the production of asphyxia: (1) atelectasis; (2) destruction of the alveolar lining cells with formation of a hyaline membrane; (3) extensive capillary damage, resulting in severe congestion, focal hemorrhage, and edema of such a degree that some animals literally drowned in their own fluid. The factor of viral intoxication was difficult to judge; one animal died in 18 hours with slight involvement of the lungs, but all other animals that died of the disease had extensive pulmonary lesions.

The experiment is summarized in Text-Figure 1 consisting of three graphs in which the processes of necrosis, proliferation, and cellular exudate are plotted against time. The abscissas show the number of days after inoculation of the virus, while the ordinates indicate the severity of the process, ranging from 1 plus to 6 plus. The severity was measured subjectively. Therefore the curves are to be considered as diagrammatic and as representing trends rather than exact mathematical replicas of the processes.

The formation of the pink-staining hyaline membranes in the bronchioles and alveoli was clearly on the basis of necrosis of the lining cells. The membranes were formed by a fusion of these necrotic cells and one could often see nuclear remnants within them (Figs. 4 to 7). They could hardly have been produced by the packing of fibrin into the alveoli since there was no fibrin in the alveolar exudate.

As to the etiology of the pneumonia, it seems quite clear that the only significant etiological agent was the virus itself. The few bacteria introduced into the lungs, either by their presence in the inoculum in one case (virus-lung suspension) or by their being washed down from the nasopharynx in the other (virus-egg suspension), played no part either in the pathogenesis of the pneumonia at the onset or in the production of a secondary bacterial pneumonia. In the former group, bacteria were cultured from the lungs in the first 48 hours only, and, in the

latter, in the first 24 hours only. Thereafter, the cultures of the lungs were negative and the pneumonia progressed to its peak on the 4th day in the absence of bacteria. No bacteria were seen in the specially stained sections of lung in either group. It was seen that the intranasal inoculation of about 25 million Salmonella or *B. coli* organisms produced only



Text-Fig. 1. The three main processes of epithelial necrosis, epithelial proliferation, and cellular exudation are represented in these graphs. The abscissas show the number of days after inoculation of the virus and the ordinates the estimated intensity of the processes. These curves are purely diagrammatic.

a slight degree of interstitial mononuclear reaction in the lungs; it is quite improbable that such a large number of bacteria were washed down with the virus suspension. Although small numbers of non-hemolytic streptococci were present in the virus-lung inoculum, it seems reasonably certain that they were of no importance in the pathogenesis of the pneumonia, since no cocci were seen in the sections of lung stained for bacteria.

The lesions described in this experiment are essentially similar to those produced in mice by Straub [4, 5] with the viruses of swine and human influenza, and by Nelson and Oliphant [6] with virus A of human influenza and Oliphant and Perrin [7] with virus B of human influenza. Straub described the bronchi and lungs only, while the others performed complete autopsies on the mice and found no important lesions outside of the trachea, bronchi, and lungs. In the present experiment, also, lesions were restricted to the trachea, bronchi, and lungs.

The nose of the mouse, unlike that of the ferret,[10] suffers no lesions from the introduction of the viruses of human and swine influenza.

No lesions were found in the brain in this experiment. Nelson and Oliphant [6] and Oliphant and Perrin [7] also found no lesions in the brains of mice given intranasal inoculations of the viruses of human influenza. Sheftel,[11] on the other hand, described changes in the brains of mice receiving intranasal inoculations of the Leningrad strain of the virus of human influenza.

The anatomical changes in the respiratory tract of mice, produced by the viruses of swine and human influenza, are quite similar to those of certain types of pandemic influenza-pneumonia in man described by Winternitz, Wason, and McNamara [12] and by Goodpasture,[13] and considered by Goodpasture probably to be of viral origin.

There has been considerable controversy as to whether the epithelial lining of the alveoli in chronic pneumonias is derived from the bronchioles or from the alveoli. In the present experiment I have gained the impression that the filling of the alveoli with hyperplastic epithelium was mainly the result of the downgrowth of such epithelium from the bronchioles. At the same time there was a slight degree of proliferation of the cells lining the alveoli. Whether or not these cells were epithelial in nature was not determined; at least they closely resembled the hyperplastic cells in the bronchi and they did not appear to be phagocytic.

Many of the solid plugs of epithelium in the alveoli underwent degeneration between the 2nd and 3rd weeks, resulting in partial reopening of some alveoli. On the other hand, Straub [5] has shown that some of these epithelial plugs persist for a year or even longer.

Summary

The intranasal inoculation of mice with the virus of swine influenza produced necrosis of the lining of the bronchi and alveoli and to a lesser extent of the lower portion of the trachea. The necrotic epithelium often appeared in the form of a hyaline membrane. Even before the necrotic process had ended, proliferation of the epithelium began and reached a remarkable degree in the bronchi and bronchioles. From the latter the proliferating epithelium invaded and filled the alveoli. Many of these intra-alveolar plugs of epithelium apparently began to degenerate about the 14th day and some alveoli were thus partially reopened. The epithelium of the bronchi and bronchioles was restored to normal sometime during the 3rd week.

In addition to bronchitis, there was a pneumonia characterized by hyaline necrosis of alveolar cells, congestion with focal hemorrhage, marked edema, and interstitial infiltration of inflammatory cells, chiefly mononuclear cells. Large areas of lung collapsed as early as the 2nd day, subsequent to obstruction of bronchi by pus, mucus, and desquamated cells. Most deaths occurred on the 4th day, mainly from asphyxia resulting from the pulmonary changes.

Lesions were restricted to the trachea, bronchi, and lungs. In particular, no changes were found in the nose or brain.

The lesions were essentially similar to those reported by others as occurring in mice following the intranasal inoculation of the virus of human influenza.

The photomicrographs were made by Mr. Carl M. Bishop.

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[*Illustrations follow*]

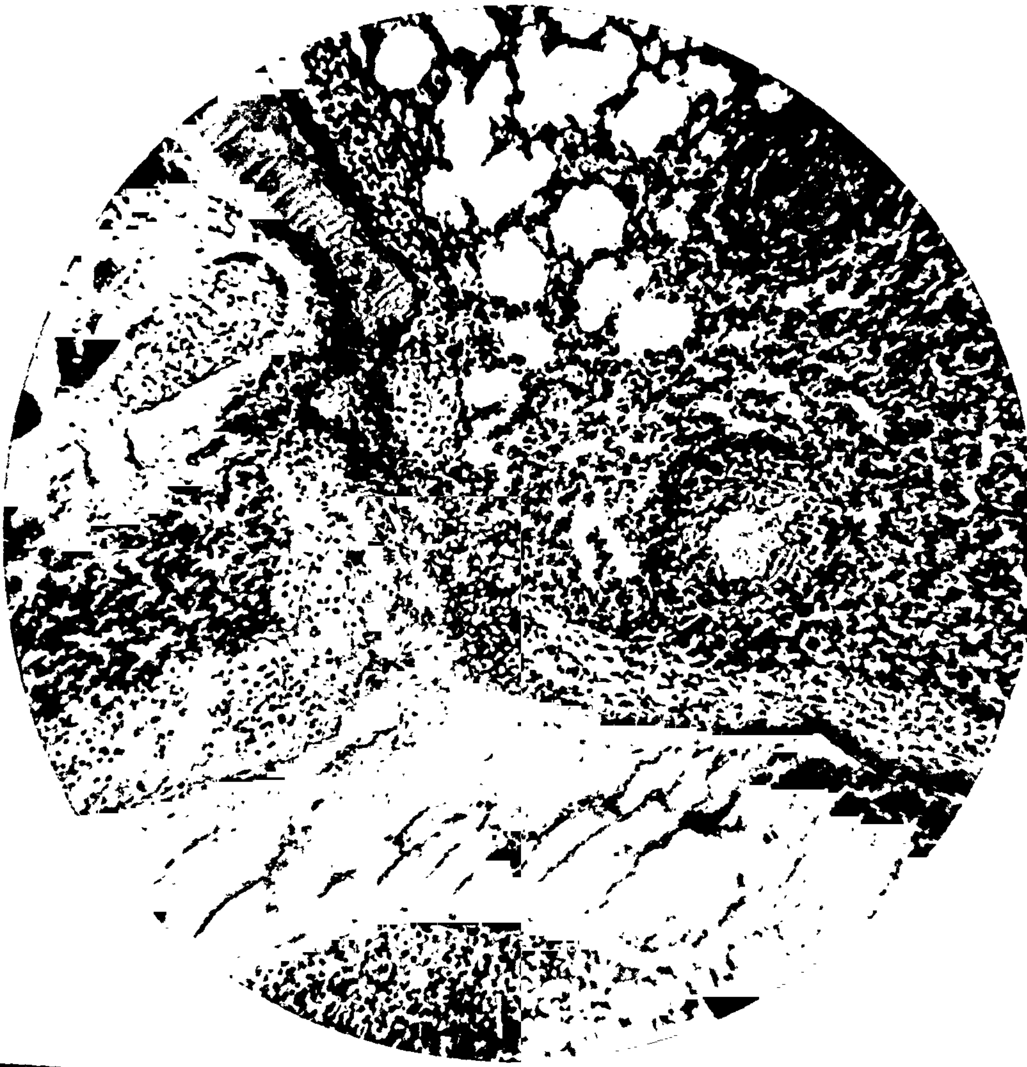
DESCRIPTION OF PLATES

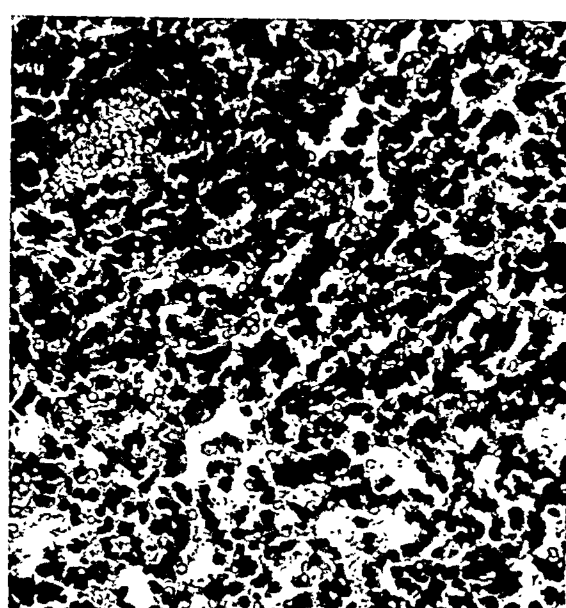
PLATE 197

FIG. 1. Microscopical appearance of the lung of a mouse 2 days after intranasal inoculation of the virus of swine influenza. A bronchus in the upper left portion of the field is plugged with exudate; it is such occlusion of bronchi which leads to atelectasis, an area of which is seen at the extreme right. Also of note are the perivascular edema and cellular infiltration. Hematoxylin and eosin stain. $\times$ 122.

FIG. 2. Twenty-four hours after inoculation of the virus, the lumen of a small bronchus is filled with pus and desquamated necrotic epithelium. Hematoxylin and eosin stain. $\times$ 684.

FIG. 3. Diffuse interstitial mononuclear infiltration of atelectatic lung on the 5th day. Hematoxylin and eosin stain. $\times$ 265.





Dubin

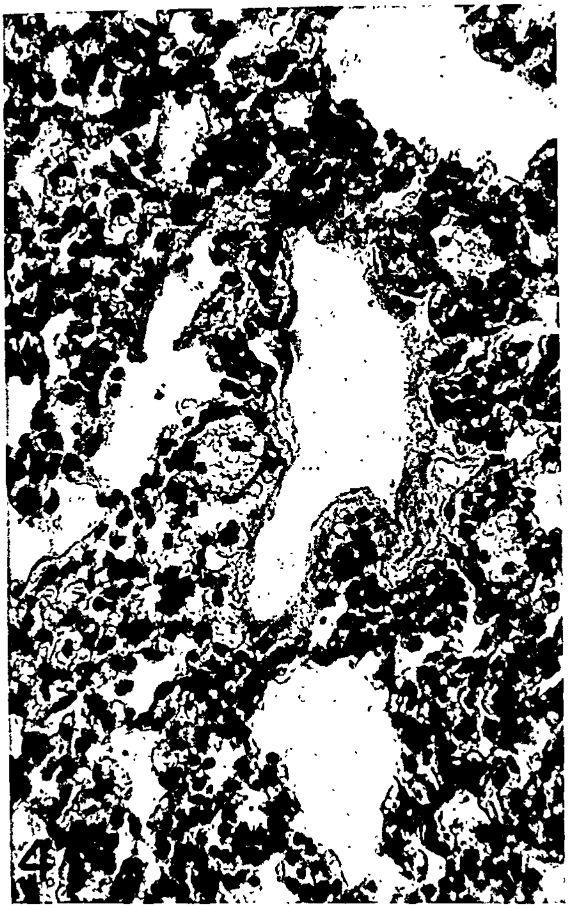
Mice Infected with Swine Influenza

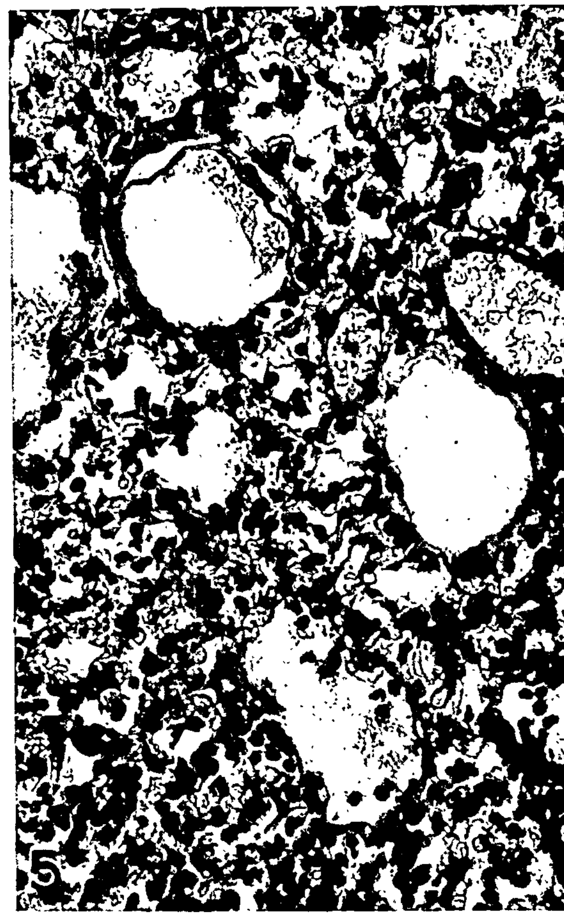
PLATE 198

FIGS. 4 and 5. Hyaline membranes lining bronchioles, alveolar ducts, and alveoli; 6th day. Hematoxylin and eosin stain. × 275.

FIG. 6. Hyaline membranes in three adjacent alveolar ducts arising from one bronchiole. Damaged cells with pyknotic nuclei make up the membranes. The bronchiole is lined by a single layer of hyperplastic polymorphic cells; 5th day. Hematoxylin and eosin stain. × 265.

FIG. 7. High-power view of a portion of Figure 6 showing the formation of the membrane from necrotic epithelium. Hematoxylin and eosin stain. × 684.





Dubin

Mice Infected with Swine Influenza

PLATE 199

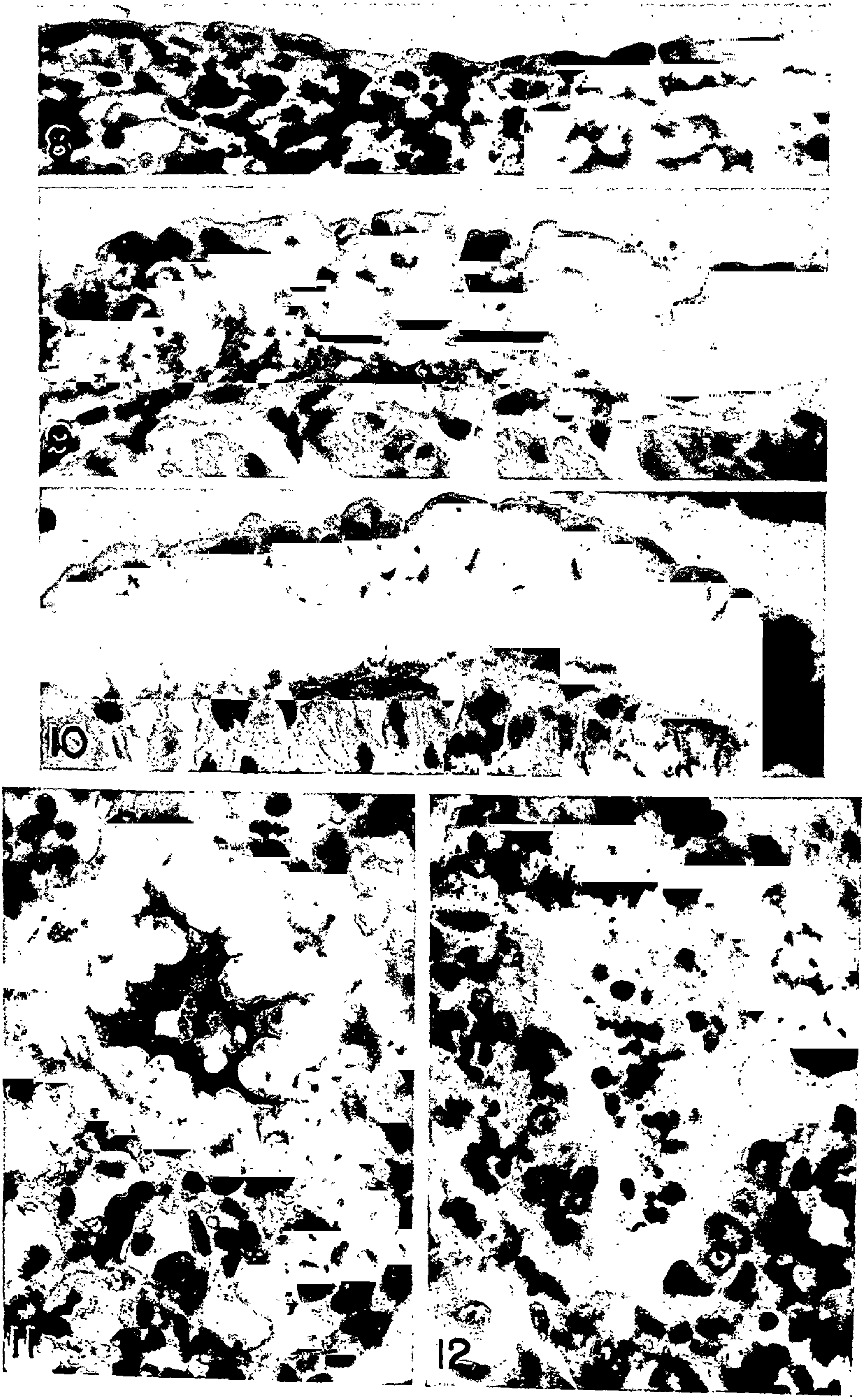
FIG. 8. Three days after inoculation. A large bronchus is now lined by a single layer of flat cells. Hematoxylin and eosin stain. $\times$ 684.

FIG. 9. Two days after inoculation. Proliferation of the lining epithelium of the trachea. In most instances the trachea did not show as much proliferation as this. Hematoxylin and eosin stain. $\times$ 684.

FIG. 10. Four days after inoculation. Proliferation of the lining epithelium of a large bronchus. Hematoxylin and eosin stain. $\times$ 684.

FIG. 11. Five days after inoculation. Proliferation of the epithelium of a bronchiole. Hematoxylin and eosin stain. $\times$ 684.

FIG. 12. Seven days after inoculation. A small bronchus is lined by hyperplastic epithelium and its lumen still contains débris. Hematoxylin and eosin stain. $\times$ 684.



Dubin

Mice Infected with Swine Influenza

PLATE 200

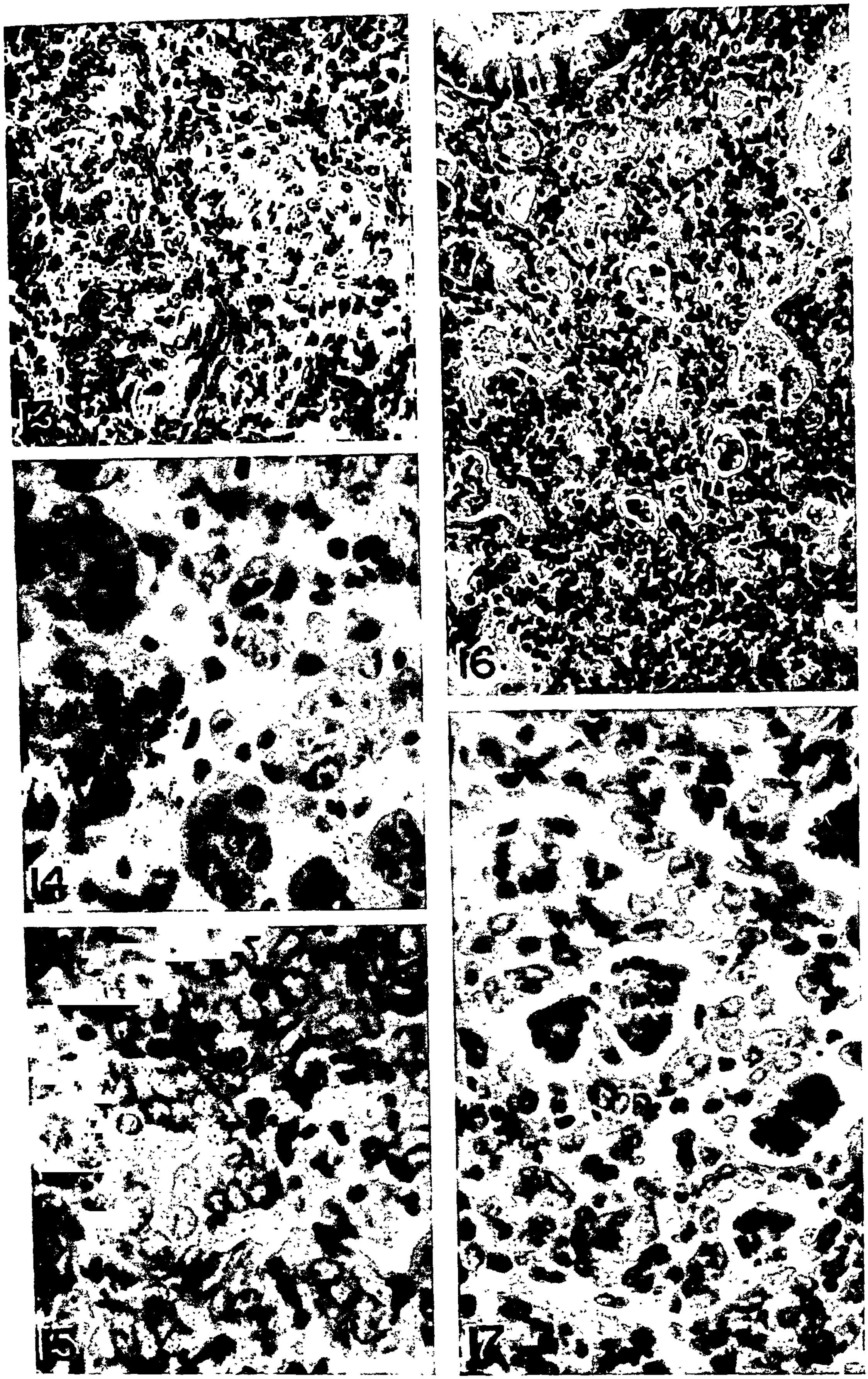
FIG. 13. Twelve days after inoculation. The alveoli are filled with epithelial plugs, some of which resemble squamous epithelium. Hematoxylin and eosin stain. × 265.

FIG. 14. High-power view of a field adjacent to that seen in Figure 13. Hematoxylin and eosin stain. × 684.

FIG. 15. Fourteen days after inoculation. The nuclei of the cells forming the epithelial plugs are swollen. This will be followed by necrosis and desquamation of these cells and by partial reopening of some alveoli. The appearance of these cells may be compared with those in Figure 14 photographed at the same magnification. Hematoxylin and eosin stain. × 684.

FIG. 16. Nineteen days after inoculation. The alveolar ducts and alveoli are filled with hyaline eosinophilic masses consisting of degenerated epithelial cells. The interstitial tissue contains mononuclear cells. The bronchus in the upper left portion of the field still contains fluid and débris; the epithelium is relatively normal. Hematoxylin and eosin stain. × 265.

FIG. 17. Twenty-three days after inoculation. The lung has the general appearance of a gland. The alveoli are lined by cuboidal epithelium. The hyaline masses in the alveoli are surrounded by polymorphonuclear leukocytes. Hematoxylin and eosin stain. × 684.



Dubin

Mice Infected with Swine Influenza

PRIMARY SPLENIC NEOPLASMS *

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Splenic tumors were originally classified by Weichselbaum [1] in 1881 into three groups: spindle cell sarcoma, endothelial sarcoma, and lymphosarcoma. In 1923, Smith and Rusk [2] placed all primary splenic tumors as derivatives of the (a) capsule or trabeculae, *i.e.*, fibrosarcoma, (b) lymphoid tissue, and (c) vascular and sinus endothelium. Upon examining the literature, it immediately becomes apparent that these limited groups fail to include all of the types which have appeared in the 157 reported primary splenic tumors (42 since Howard's [3] report of 115 in 1929). Consequently, it becomes necessary to classify splenic tumors in accordance with all of the different cell types found in the spleen. From this it follows that there are seven fundamental neoplastic types, six of which have been reported. These seven are derived as follows: (1) vascular elements—angioma; (2) lymphoid tissue; (3) reticulo-endothelial cells—endothelioma and reticulum cell sarcoma; (4) embryonic inclusions—dermoids, epithelial cysts, and mesothelial inclusion cysts; (5) fibrous tissue derivative—fibrosarcoma and fibroma; (6) smooth muscle—leiomyoma; (7) nerve elements—neuroma or neurosarcoma.

Judged on a basis of 40 reported cases, the nonspecific diagnosis of splenic sarcoma is most frequent, and hemangioma next most frequent. The different types of reticulo-endothelioma and lymphosarcoma follow and are practically equal in number. Lymphangiomas are slightly less frequent, and the remaining types are relatively infrequent. As yet, neurosarcoma of the spleen has not been reported. Krumbhaar [4] reported that 0.64 per cent of all tumors of the body are primary in the spleen, and Lubarsch [5] found 3 angiomas in 19,000 autopsies. As to the splenic tumors which I am reporting in this article, two endothelial sarcomas, the reticulo-lymphosarcoma, the reticulum cell sarcoma, two angiomas, and the epidermoidal cyst were encountered in a series of 11,707 autopsies and 68,820 surgical specimens. Three cystic splenic tumors were encountered during this period, but these are not discussed on this occasion.

Lymphoid Tumors

The age distribution for the lymphoblastic tumors of the spleen ranged from 11 to 75 years in the reported cases. Most of the spleens weighed over 1000 gm. and were often nodular. In some cases these

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resembled a "sago spleen" and in others the nodules were up to 4 cm. in diameter. In the instances of rather diffuse nodularity, differences of opinion exist on very similar cases, as, for example, Ross' [6] diagnosis of lymphoid reticulosis of a nonneoplastic type which, in a very similar instance, was diagnosed by McNee [7] as lymphosarcoma. Frequently these patients suffer from an anemia of a secondary type, and, having also splenomegaly, are often placed clinically in the category of Banti's syndrome.

Microscopically, the essential cell type is the lymphocyte, which tends to appear somewhat immature, but may not be. Authors frequently refer to a slightly increased amount of cytoplasm over that which would be found in a lymphocyte in the blood. Mitotic figures are abundant, and the cells may lie on a reticular stroma but do not produce it. In the small-celled type giant cells are not found typically, but as the size of the cell increases to that of the large lymphocyte or lymphoblast, multinucleated forms are more likely to be found. Warren and Picena [8] proposed that when appreciable lymphopoiesis occurs the tumor should be called a reticulo-lymphosarcoma; when it is absent, a reticulum cell sarcoma.

Under lymphoid tumors in general, Hodgkin's granuloma can probably best be discussed. As a secondary tumor of the spleen it is quite common, but as a carefully proved primary lesion it is distinctly rare. Cases have been reported by Symmers,[9] Wade,[10] and Mellon.[11] Ewing [12] warned that many supposed Hodgkin's tumors will be found to belong correctly among other splenic neoplasms, probably most frequently the pleomorphic group of endotheliomata.

Tumors Derived from Reticulo-Endothelial Cells

PRIMARY RETICULO-LYMPHOSARCOMA

Report of Case

Clinical Résumé. M. M. (no. CO-42-1313) was a white female, 90 years old. She had been in the hospital three times during the past 8 years, each time for degenerative vascular disease. Blood counts were always normal and lymph nodes were never enlarged. She last entered the hospital following a fall. A complete physical examination was done. No lymphadenopathy was observed, and other findings were only arteriosclerotic changes and bruises.

Laboratory examination showed the hemoglobin to be 70 per cent; red blood cells, 4.3 million; white blood cells, 14,000, with 87 per cent polymorphonuclear cells and 13 per cent lymphocytes. The patient expired. The clinical diagnosis was cerebral edema and arteriosclerotic heart disease.

A complete autopsy was done. The immediate cause of death was cerebral contusion. The spleen was four times normal size. It was diffusely adherent to the abdominal wall. On section it presented a

friable, congested parenchyma. Scattered throughout, but especially at its inferior pole, were nodules of gray, circumscribed abnormal tissue that were quite soft. They varied from 1.5 to 3.5 cm. in diameter, with the largest ones showing a confluence in the inferior pole. The abdominal cavity, liver, mediastinum, and lymph nodes were searched for other evidence of tumor formation, but none was found.

Microscopically, the tumor nodules in the spleen showed no tendency to be organoid and did not line sinuses, or form vascular channels (Fig. 1). In some areas degenerative changes were seen. The tumor cells were pleomorphic in size and type and occasionally multinucleated cells were present with oval or folded, dark nuclei. These cells had a moderate amount of cytoplasm. Also present, and comprising about 50 per cent of the cells, were round cells with indistinct outline and an oval or round nucleus without prominent nucleoli and rather uniform chromatin. Mitotic figures were quite frequent among these cells. They were about twice the size of a blood lymphocyte and resembled the basic type of that series. Finally, in the tumor there were seen scattered foci of cells possessing all of the characteristics of young lymphocytes. Search for eosinophilic leukocytes was fruitless, and special stains showed only an occasional larger tumor cell producing reticulum, although it was present in moderate amount in some areas.

Diagnosis. Primary reticulo-lymphosarcoma of the spleen.

Discussion. In view of the complete clinical study and the complete gross examination at autopsy, during which no evidence of tumor was found except that in the spleen, there remains no reasonable doubt that this is a primary splenic neoplasm. In its general pattern it is rather pleomorphic, with evidence of immaturity and with cellular proliferation of a reticular type. In addition, there is noted a significant number of round cells of the lymphocytic series and evidence of lymphopoiesis, which indicate a differentiation toward the lymphosarcoma type. With this tendency in mind, it is felt that this tumor is best placed in the group of reticulo-lymphosarcomas of the spleen.

RETICULUM CELL SARCOMA

Howard [3] and Gerundo and Miller [13] presented examples of primary reticulum cell sarcoma, and Gerundo and Miller considered that the reticulo-endothelial sarcoma reported by Langenstrass and Neumann [14] would have been best classified as a reticulum cell sarcoma. In establishing the diagnosis, the gross picture presents nothing characteristic, in that it may show a single mass in the form of a coarsely lobulated tumor, or exist as separate nodules. These nodules tend to be firmer than the surrounding spleen, and somewhat gray.

Microscopically, using the rather strict description by Oberling[15] and Warren and Picena,[8] the picture is as follows: The nuclei of the cells are irregularly distributed, oval or somewhat indented. These nuclei are quite prominent and have a well defined nuclear membrane enclosing a finely divided and scattered chromatin. In the more anaplastic forms one or two prominent nucleoli may be seen. The general paleness of the karyoplasm is often quite prominent. The cytoplasm in the undifferentiated forms consists of poorly outlined syncytial masses of undivided or slightly fenestrated protoplasm. In the more differentiated types the cell may be round, oval, or somewhat elongated, and 15 to 20 μ in diameter. The reticulum formation may vary from a few scattered, mostly intracytoplasmic, reticulum fibers to a quite complete network of reticulum passing between groups of individual cells. The cytoplasm may be rather abundant, and be either lightly acidophilic or basophilic. Occasionally multinucleated or binucleated cells are found, but these are not characteristic. Oberling stated that mitotic figures do not occur in great numbers, a conclusion quite the opposite to that of Parker and Jackson[16] and one not upheld in the reported cases. Lymphocytes may be seen in the stroma. The tumor cells should not show characteristics of tissue organization, such as actual new blood vessel formation. Some phagocytic activity of the cells may be demonstrable, but this is not considered essential for the diagnosis. In the most fully differentiated type of reticulum cell tumor, occasional areas of distinctly fusiform cells may be seen with some coalescence of the abundant reticulum fibers into strands of collagen. Such areas are not unlike fibrosarcomas, from which they may be clearly separated by examining the more undifferentiated portions of the tumor.

Gall and Mallory[17] have suggested the division of the reticulum cell sarcoma into the "stem cell" and "clasmatocytic" types. These appear to be valid subdivisions which had been recognized in the literature as variations in differentiation, but had not been individually named. This division, as applied to splenic sarcoma, needs further clarification.

Report of Case

L. A. (no. SP-44-1704) was a white housewife, 48 years of age. Her husband was living and well.

Present Illness. Five months before entry, the patient rather suddenly developed excessive malaise, dizziness, and nausea without vomiting. At that time she felt "feverish" and noted slight evening sweats. Her doctor found that her hemoglobin value was 40 per cent. He gave her iron, without effect. Two months before entry, while at rest, she suddenly developed a severe stabbing pain in the left upper quadrant which radiated to the left shoulder and was made worse with deep breathing and coughing. During 7 weeks of hospitalization, roentgenograms were made of the abdomen, and transfusions and supportive therapy were administered.

Numerous blood counts and blood cultures (all negative) and a sternal biopsy were done without establishing a specific diagnosis. While in the hospital she had daily afternoon fever of 100° F. and drenching night sweats. Petechiae were never noted. Weight loss was about 50 lbs.

Physical Examination. Ears, nose, and throat were negative on physical examination. Motion of the left half of the diaphragm was limited and the lower left pulmonary lobe showed dullness, decreased fremitus, and decreased breath and voice sounds. The heart was not enlarged, and the rhythm and rate were regular. P_2 was greater than A_2, and there was a loud, blowing systolic murmur over the precordium. Blood pressure was 110 mm. of Hg systolic, 165 diastolic. In the abdomen the upper left quadrant was tender and the spleen very large, extending to the umbilicus. The liver could not be felt, and no other masses or abnormalities were noted. Rectal and pelvic examinations were negative.

Laboratory Findings. Examination of the blood revealed the white blood cells to be 18,500, with 83 per cent polymorphonuclear leukocytes, 11 per cent lymphocytes, 5 per cent monocytes, and 1 per cent eosinophils. Red blood cells were normal and showed no sickle cells. Stains for malarial organisms were negative on four occasions. Hemoglobin, 65 per cent; red blood cells, 3.7 million. Cell volume, 34; color index, 0.74; volume index, 0.98; saturation index, 0.75; icteric index, 1.7; Rh factor, positive; red blood cell fragility, 0.48 to 0.36; Wassermann test, negative. Urine: Tests for albumin, Bence-Jones proteins, and sugar negative; occasional red blood cells and 1 white blood cell per high dry field. Serum protein total, 6.2 gm. per cent, with 3.6 gm. albumin and 2.2 gm. globulin. Agglutination tests for typhoid, paratyphoid, tularemia, undulant fever, and dysentery were negative. Sedimentation rate, 32 mm. (Wintrobe, noncorrected). Platelets, 390,000. Bleeding time (Ivy), 3 minutes. Coagulation time, 5½ minutes. Five blood cultures and a sternal culture were negative. Tuberculin tests at 1:1000 and 1:100, and a coccidioidin test were negative. Roentgenograms revealed an abdominal mass in the left upper quadrant; specific diagnosis not possible.

Course. The patient continued to have a daily fever from 38° to 39° C., with night sweats. Transfusions, sulfonamides, and penicillin were without effect. Splenectomy was decided upon, with a clinical diagnosis of probable tuberculoma of the spleen, or possible subacute bacterial endocarditis with old rheumatic heart disease and aortic insufficiency.

The spleen weighed 1450 gm. and measured 25 cm. in length. The surface was smooth and a dark slate-red. There were some irregularly shaped, pale areas on one surface. The hilar region revealed normal vessels and no neoplastic tissue. However, there were noted two small accessory spleens and four lymph nodes in this region. On section there was seen a large, central, yellowish white tumor mass (Fig. 2) occupying almost the entire organ and extending to within 3 to 4 cm. of each pole. The remaining splenic tissue existed as a rather compressed capsule 1 to 2 cm. thick. The tumor varied in consistency from a predominantly fibrous texture, through a softer sarcomatous consistency, into other areas of apparent mushy necrosis. A few small areas of cystic degeneration were noted which contained brownish fluid.

Microscopically, the predominant cell was one of moderate size with a rather abundant, pale, slightly granular cytoplasm having an indistinct cell membrane and containing a prominent, delicate, slightly

irregular oval nucleus. The nucleus had a distinct membrane, scanty chromatin, and one or several variably prominent nucleoli. Mitotic figures occasionally occurred. This rather succulent type of cell changed into a distinctly fusiform cell arranged in vague bands in some areas. In such areas reticular formation by the tumor cells was massive and collagen was evident. Elsewhere there was no collagen and, instead, the reticulum production by the cells was delicate and moderate in amount or completely absent. In some areas the tumor cells had phagocytized erythrocytes, granulocytes, or débris. There was no suggestion of formation of vascular channels by the tumor cells. In the background scattered lymphocytes and many eosinophilic leukocytes were noted. In many areas tissue degeneration with loss of all cellular detail was evident. The surrounding splenic tissue contained many red corpuscles, polymorphonuclear and lymphocytic leukocytes, and many eosinophilic granulocytes. The germinal follicles were of fairly normal appearance and the capsule was negative (Figures 3 to 5).

The sections of the hilar lymph nodes and accessory spleens showed no neoplastic tissue and a pattern that was normal.

Diagnosis. Primary reticulum cell sarcoma of the spleen.

Discussion. The moderate-sized, often rather syncytial-appearing cells, showing delicate reticulum production and scattered evidence of phagocytosis, point distinctly toward the reticulum cell origin of this tumor. In certain areas this tumor becomes highly "differentiated" (Oberling [15]) and assumes a fusiform cellular pattern together with heavy reticulum production and collagen. In such areas the cells have differentiated toward fibroblasts and the tumor is not unlike a fibrosarcoma. The term reticulo-fibrosarcoma has been suggested for such a tumor. Perhaps as a descriptive phrase this may be justified. However, such a term is not established in the literature and certainly Oberling's class of "differentiated reticulum cell sarcoma" more nearly expresses the correct cytological derivation of these tumors.

ENDOTHELIAL SARCOMAS

In the literature, splenic endotheliomas are next to, or equal to, lymphosarcomas in frequency. In six carefully studied cases obtained from different reports in the literature, the ages ranged from 33 to 67 (average, 52 years), the incidence of male to female was equal, and the spleens weighed from 420 to 5300 gm. with an average of 1700 gm. Although it is one of the more frequent tumors of the spleen, Ewing [12] remarked that in the spleen, as in lymphoid tissue in general, endothelial cells tend to lack many of their classical characteristics and that tumors derived from them are considered subvarieties of sarcoma.

For that reason a diagnosis of endothelioma should be carefully established, and this group must not serve as a ragbag for poorly defined tumors.

Connor,[18] in his study of marrow tumors of the endothelioma group, formulated three fundamental types which correspond well with similar tumors found elsewhere in the body. The first type is the angio-endothelioma, characterized by the differentiation toward vascular tissue with formation of sinuses and vascular spaces. The second type is the diffuse tumor in which the cells appear in sheets and masses, forming no definite structure, but sometimes, and in some areas, the cells are in rows or small alveoli. The third group is designated the reticular type in that its cells are united by fine protoplasmic processes and there is a variable amount of reticulum production. This form is usually regarded as a more primitive type, intermediate between the first two forms. This general classification applies very well to the endothelial tumors of the spleen and lymphoid tissue in general. It follows, therefore, that the establishment of the identity of a mesenchymal tumor of the spleen would be greatly aided by the discovery of the formation of sinuses, alveolar spaces, or a radiating perivascular arrangement.

Specific cytological details of the endothelioma of the spleen have not been established in the literature, probably because of the rather wide variation which is found. However, a composite picture of the cell type as found in a series of well authenticated cases should serve as a base line for classification. The cell tends to be rather large, some 20 to 30 μ in diameter. Its outline, in areas showing little or no structural differentiation in the direction of lining tissue spaces, is usually round or oval, and in more fibrous-appearing areas may be elongated. The cytoplasm is rather pale or lightly acidophilic, and tends to be not very abundant. The nuclei are prominent, usually oval, less frequently lobulated, and with a distinct nuclear membrane. A finely reticulated karyoplasm contains some dark chromatin particles and usually one or two eccentrically placed, dark nucleoli. Multinucleated giant cells in moderate or occasionally fairly large numbers are usually found, and mitotic figures are characteristically frequent. Phagocytosis by the tumor cells may be seen, but this does not differentiate them from reticulum cells or mesenchymal cells in general. It will be noted that in the description of the nucleus there is a marked difference from the typical endothelial cell. In the exceptional tumor, the delicate, non-nucleolated classical type of endothelial cell will be found. Ewing [12] recognized this difference in endothelioma of the lymphoid system.

From this cytological description, it can be appreciated that the real problem in the classification of splenic tumors lies between the

reticulum cell sarcoma and the diffuse endothelial sarcomas producing reticulum (reticulo-endothelial sarcomas). Unless one wishes to accept the rather strict criteria for reticulum cell sarcoma, as proposed by Oberling [15] and Warren and Picena,[8] this separation probably cannot be made consistently.

PRIMARY ENDOTHELIOMA

Report of Cases

Clinical Résumé. C. W. (no. SS-41-1066) was a white switchman, 57 years old. He was entirely well until 2 weeks before entry, at which time he began to notice crampy, left-sided pain after eating, some nausea, but no vomiting. There had been no weight loss. Physical examination revealed no abnormalities except those referable to the abdomen. This presented generalized tenderness and rebound tenderness in the left lower quadrant. The general picture was that of an early, generalized peritonitis.

Examination of the blood showed: hemoglobin, 80 per cent; red blood cells, 3.8 millions per cmm.; white blood cells, 24,000 per cmm. with 90 per cent polymorphonuclear leukocytes; platelet count, 150,000 per cmm.; clotting time, 10 minutes, bleeding time, 2.5 minutes; Wassermann test, negative, nonprotein nitrogen, 27 mg. per cent.

At operation, a considerable amount of free blood was seen in the peritoneal cavity, and the spleen was found to be torn at one pole. The spleen was removed. No other abnormalities were encountered. The patient made an uneventful recovery, and further study revealed no evidence of a lymphomatous tumor in the lymph nodes.

The spleen weighed 455 gm. The external appearance was normal except for a rupture at one pole. This was in the form of a hole that measured 1 by 1.5 cm. Upon section, it was seen that this rupture had occurred in a tumor measuring 3 by 4 cm. in cross section. This tumor was pink-white, soft to palpation, and with no gross evidence of necrosis except at the point of rupture. The tumor was quite distinct from the rest of the spleen and there were a few small satellite tumors immediately surrounding the main mass. A small accessory spleen also was removed.

Microscopical examination showed a capsule which, where it was not broken, was well formed. The splenic tissue revealed compression and thickening of the trabeculae, but congestion was not prominent and the malpighian corpuscles were normal. The neoplastic tissue was clearly separated from the surrounding splenic tissue. The tumor pattern was somewhat distorted by the presence of an inflammatory reaction. Nonetheless, there was a distinct picture of tumor cells lying attached to each side of delicate, irregular, but roughly parallel connective tissue septa, and projecting into the intervening spaces (Fig. 6). In some areas this property of lining tissue spaces was more prominent than in others. The neoplastic cells revealed some pleomorphism, and were of moderate size, being from 21 to 28 μ in

diameter. The cytoplasmic outline was indistinct, usually rather irregular but occasionally round. The cytoplasm was pale and not abundant. The nuclei were oval or rounded and occasionally slightly folded. The nuclear membrane was distinct and moderately heavy. The karyoplasm was rather pale and contained scattered and somewhat coarse chromatin with usually one rather prominent, slightly eosinophilic nucleolus. A moderate number of multinucleated tumor cells were seen, and mitotic figures and pyknotic nuclei were frequent. In many areas there was a diffuse polymorphonuclear leukocytic infiltration which was more abundant in and near areas of hemorrhagic degeneration. Associated with this was a scanty but evident eosinophilic leukocytic infiltration. Lymphocytes were not seen except where left behind by an invaded splenic pulp. Silver stains showed throughout an increased amount of reticulum, which was intercellular in distribution and which was not seen to arise within the tumor cells.

Diagnosis. Primary endothelioma of the spleen, with focal necrosis and rupture.

Discussion. The inflammatory reaction somewhat confuses the microscopical appearance of this tumor because of the distinct infiltration of eosinophils, which superficially suggests a Hodgkin's type of reaction. However, it is felt that this is purely an inflammatory reaction. In addition the tumor pattern lacks the background of fibrosis and varying cellular types found in Hodgkin's disease. The formation and lining of tissue spaces by the tumor cells is distinct, and somewhat resembles the lining of the splenic sinusoids, in that the tumor cells lie on either side of delicate connective tissue strands. With their moderate neoplastic pleomorphism, these cells are in distinct contrast with the cells found in the primary splenic endothelioma of the case described below. In the latter the tumor cells more closely resembled the standard accepted type for endothelial cells. Such is usually not the case, for the primary splenic endotheliomata more frequently present the pleomorphic pattern of the tumor presented by this patient.

Clinical Résumé. E. J. (no. SS-41-1100) was a white housewife, 55 years old. Ten years before her present hospitalization a mass was noted in the abdomen, and after careful clinical investigation was diagnosed as most probably a cyst of the kidney. At operation a large cyst was found behind the stomach. This arose from the region of the tail of the pancreas and was retroperitoneal. The spleen and kidneys appeared normal to inspection and palpation. The cyst was evacuated and a specimen was taken for biopsy. The pathological diagnosis was fibrous tissue without epithelium and showing chronic inflammation. The surgical diagnosis was pancreatic cyst.

The present entry into a hospital was occasioned by the onset of convulsive seizures or "spells" which, even after careful investigation, were not identified as to etiology. In the hospital these increased gradually in frequency in spite of therapy, and the patient went progressively downhill to her death.

Physical examination revealed no abdominal masses, although urograms showed some calcification in the region of the spleen or pancreas. Laboratory reports showed a drop in the hemoglobin from 82 to 50 per cent, with a corresponding drop in red blood cells from 5.0 to 3.2 million. White blood cells dropped from 12,000 to 7,000, with 70 per cent polymorphonuclear leukocytes, 20 per cent lymphocytes, 2 per cent eosinophils, 4 per cent monocytes, and 1 per cent basophils. The blood smear was normal, and platelets were numerous. The Wassermann test was negative and the glucose tolerance curve normal. The serum calcium was 8.3 mg. per cent, and nonprotein nitrogen, 32 mg. per cent.

A complete autopsy was done, and the essential findings were bronchopneumonia and a tumor of the spleen. There was no evidence of metastases. The spleen weighed 1340 gm. and measured 19 by 16 by 13 cm. One pole was occupied by a nearly round tumor mass, 12 cm. in diameter. The capsule of the tumor was quite dense and in some places contained calcified material. In the center of the tumor, degeneration was seen.

On microscopical examination (Figs. 7 and 8) the splenic tissue showed a normal capsular and trabecular pattern. Splenic sinusoids and pulp tissue contained no abnormal cellular types and lacked unusual congestion. The malpighian bodies were discrete and of moderate size. The arterioles showed thickened walls, but no perivascular fibrosis. In some sections the splenic tissue ended abruptly at points of neoplastic invasion. The tumor had invaded the spleen and also the muscles of the abdominal wall where the tumor had become adherent to the wall.

The essential pattern of the tumor was one of a rather delicately fenestrated mass of relatively uniform cells, which might assume an alveolar pattern or which might be arranged along, and line, distinct clefts in the tumor. This general pattern might be distorted by areas of degeneration, containing calcium salts and cholesterol crystals, with loss of cellular structure except around larger vessels, where a peritheliomatous arrangement of the neoplastic tissue was striking.

The tumor cells were fairly uniform in type and size. The majority were round or oval, but might be elongated in areas of distortion or degeneration. The cytoplasm was lightly acidophilic, only moderate in amount, very poorly outlined, and frequently flowed imperceptibly into adjacent stellate tumor cells. This syncytial appearance was frequent, but giant cells were not seen. The nuclei were usually 10 to 14 μ in diameter, and oval. The nuclear membrane was distinct, but not coarse, and enclosed a gray nucleoplasm of ground-glass appearance containing chromatin dust and practically never any prominent nucleoli. Mitotic figures were rather infrequent. Silver stains showed only a very rare tumor cell producing reticulum. In areas of old

hemorrhage the neoplastic cells had occasionally phagocytized some iron pigment.

Diagnosis. Primary endothelioma of the spleen.

Discussion. This tumor presents no problem in classification, in that it shows certain differentiating characteristics. It obviously arose from the spleen, and the opportunity to observe the spleen 10 years before is rather unusual. The growth energy of the tumor was of a rather low order as evidenced by calcification and infrequent mitotic figures. The distinctly endothelial characteristics of the tumor cells are infrequently encountered in an endothelioma of the spleen, in which more bizarre patterns are common.

Benign Lymphangiomas and Hemangiomas

The reported incidence of benign lymphangiomas and hemangiomas varies rather widely, in that Lubarsch [5] found only 3 cases in 19,000 autopsies, yet Schottenfeld and Wolfson [19] reported an incidence of 0.14 per cent in 2,800 autopsies and Pines and Rabinovitch [20] 0.16 per cent in 3,676 consecutive autopsies. These latter authors [20] found 36 reported examples of true benign hemangioma in the literature up to 1941 and added 6 more cases of their own. The 2 reported in this paper bring the total to 44. In 1938, Akcakoyunlu [21] was able to find only 21 acceptable tumors of this type.

The average age of the patients with these tumors is from 35 to 45 years, with extremes of 4 months to 72 years. The incidence in males and females is about equal. The largest tumor reported weighed 7240 gm. (Akcakoyunlu [21]). The smaller ones are usually found incidentally at autopsy and are entirely silent clinically. Those surgically removed have usually caused symptoms and are larger. In 16 cases involving rather large tumors, Schottenfeld and Wolfson [19] listed findings as follows: abdominal tumor, 100 per cent of cases; pain, 62 per cent; anemia, 12 per cent; ascites, 12 per cent; and weight loss, 18 per cent. Except for anemia in some cases, there are no characteristic changes in the hemogram.

It is probable that the majority of these tumors arise on the basis of a congenital nevus, and slowly enlarge over a period of years. Active proliferation is difficult to establish except in the more aggressive examples. Usually physical factors dependent on congestion and hemorrhage play a prominent part, and occasionally involution may occur through thrombosis and fibrosis.

In classification, these tumors readily fall into the standard nomenclature of angiomas in general, including capillary, cavernous, telangi-

ectoides (or cystic), and mixed types, depending on the average size and dilatation of the tumor spaces. It must be emphasized that the predominant feature in these tumors is the formation of vascular channels, and not the cells involved in their formation. When it is evident that the main element consists of masses of cells which line undifferentiated vascular spaces, the tumor is essentially an endothelioma, and is properly designated a hemangio-endothelioma.

HEMANGIOMA

Report of Case

J. G. (no. A-24-205) was a Mexican, 21 years old. A splenic tumor was found incidentally during a routine autopsy, the patient having died of subacute disseminated pulmonary tuberculosis.

On gross inspection, the spleen was slightly increased in size, had a moderately firm pulp appearing to be only slightly congested. There was no gross evidence of tuberculous involvement. On section there was noted a rather soft peripherally placed area, measuring 2 cm., which was somewhat raised and darker than the rest of the tissue. It was seen to consist of several, small, cystic cavities filled with blood in an area which was not infarcted. It was interpreted as a hemangioma grossly. Microscopically, it was found to be formed of simple blood-filled spaces lined by flat endothelial cells.

Diagnosis. Hemangioma of the spleen (cavernous type).

LYMPHANGIOMA

Report of Case

C. M. (no. SA-35-166) was a white male, 35 years old. A splenic tumor was found incidentally during a routine autopsy, the patient having died of a ruptured luetic aortic aneurysm.

The spleen was of normal size and of firm consistency with a reddish purple color. The malpighian corpuscles were easily seen. At one pole there were seen a few, small, cystic structures each measuring 2 to 3 mm. in diameter.

Microscopically, the cystic structures were seen to be of variable size (Fig. 9) and to be filled with a pink, serous-appearing fluid containing no erythrocytes. Some spaces were fused with broken septa floating in their common lumen. They were lined with a single layer of low cuboidal, inactive endothelium beneath which was a thin connective tissue layer abutting directly on splenic tissue. The splenic pulp throughout was rather congested, but otherwise this tissue was normal. The arterioles showed no fibrosis. They were delicate with only slight intimal thickening.

Diagnosis. Lymphangioma of the spleen (cavernous type).

Fibromas and Fibrosarcomas

It was the fibrosarcoma which Weichselbaum [1] described in 1881 that prompted him to add spindle cell sarcoma to his other two basic types of sarcoma of the spleen: the lymphosarcoma and the endothelio-sarcoma. Since his report only two other examples of this tumor have been reported, one by Jepson and Albert [22] and the other by Heinricius.[23] They are similar microscopically to fibrosarcoma found elsewhere in the body, and consist of spindle cells predominantly arranged in fasciculi and sheets with only occasional oval-shaped tumor cells. Production of intercellular collagenous material is noted in the more differentiated areas, and the formation of vascular channels by the tumor is not seen. The occasional well differentiated reticulum cell sarcoma, which in a few areas may be spindle-celled and produce dense reticulum together with some collagen, must not be confused with the fibrosarcoma. The resemblance between the two is only superficial and by examining the more undifferentiated areas the identity of the separate cell types is apparent. Heinricius' case showed myxomatous degeneration in some areas.

Although Krumbhaar [4] stated that simple fibromas are found in the spleen, as they doubtlessly are, no example of a reported case could be found in the literature, which fact probably speaks more for their insignificance than their rarity.

Leiomyosarcoma

The single reported example of a primary leiomyosarcoma of the spleen in any animal occurred in a bovine, and was described by Feldman.[24] It had all of the characteristics of this neoplasm as it occurs elsewhere in the body, and was carefully studied by differential stains.

Dermoid and Epidermoid Cysts

Weil, Roux-Berger, and Scemama [25] have pointed out the two possible derivations of dermoid and epidermoid cysts of the spleen. The dermoid tumors and the distinctively epithelial cysts are satisfactorily explained on the basis of congenital epithelial inclusion. Other less distinctively epithelial linings probably represent metaplasia of mesothelial or endothelial-lined spaces. These authors used the term "epithelial cysts" rather loosely, including those which are apparently only metaplastic in origin. Their example possessed no distinctive epithelial characteristics other than being lined with several layers of simple squamous cells in a few areas. They accepted as epithelial cysts those with practically no cellular lining, emphasizing the apparent absence of epithelial lining cells in the obviously epidermoid cyst

of the spleen reported by Kumaris [26] which contained hair and "dermoid balls."

Keratinization, hair, and epidermal glands constitute conclusive evidence. Lacking these, intercellular bridges, pigmentation, and multiplicity of cell layers are highly suggestive, if at least two of them are present. In tumors containing multiple cysts in which only one or two of the cysts appear epithelial, the rest being structurally mesodermal, probably the apparent epithelium is due to mesodermal metaplasia. Herein lies the importance of observing the above-described criteria. In the absence of keratinization, hair, or epidermal glands, simple metaplasia cannot be positively ruled out. From the behavior of the lining cells of the female genital system it is obvious that mesodermal derivatives possess the property of forming a morphologically typical endodermal or ectodermal epithelium. This may partly account for Custer's [27] conclusion that epidermoid cysts of the spleen are not rare in that he encountered 5 cases in 5,000 autopsies. Every effort should be made to establish the origin of the lining cells of each splenic cyst under consideration.

In the literature, definitely epidermoid splenic cysts were reported by Andral [28] and by Kumaris,[26] in that they contained hair and keratin. Velasco Suarez and Angel Etcheverry [29] reported a dermoid cyst of the splenic hilum (not in the spleen) associated with hemolytic icterus and anemia. Other authors have reported splenic cysts as epidermoid in origin on the basis of squamous epithelium, at points multilayered, and almost always having intercellular bridges. Such reports were made by Weil, Roux-Berger, and Scemama,[25] Carnett, Bates, and Linney,[30] Shawan,[31] Lereboullet, Grégoire, Bernard, and Ibarran,[32] Dinand,[33] and by Montgomery, McEnery, and Frank.[34] These last authors reported 2 cases. The case presented in this paper brings the total number of reported cases of apparently epithelial-derived tumors and cysts of the spleen to 11.

All of the primary carcinomas of the spleen in the literature were reported during an era when there was still technical and morphological confusion between certain types of carcinoma and sarcoma. Therefore they were all probably sarcomas, erroneously diagnosed as primary carcinoma. However, there is always the possibility that one of these may have developed from an aberrant epithelial remnant. No such example could be found in the literature.

EPIDERMOID CYST

Report of Case

D. R. (no. SP-38-1340) was a white school girl, 14 years old. She had noticed a swelling in the left upper quadrant for the past 1½ years, which at first gave no symptoms. Later, and at intervals of several months, she would experience severe

localized abdominal cramping pains, that were unassociated with clinical signs and which would subside within a week. Physical examination was negative except for the presence of a large, rather hard, smooth mass in the region of the spleen. It was not fixed; it extended to the midline, and was 4 fingersbreadth below the left costal margin.

Laboratory work showed a normal phenolsulfonphthalein excretion and Addis count. Examination of the blood showed the hemoglobin to be 83 per cent, with 4.0 million red blood cells. White blood cells were 8,800, with 71 per cent polymorphonuclear leukocytes, 23 per cent lymphocytes, 4 per cent eosinophils, and 2 per cent monocytes.

The clinical diagnosis was probable renal tumor, and an exploratory laparotomy was performed during which a large spleen was removed. The patient made an uneventful recovery.

The spleen measured 20 by 12 by 7 cm. The greater part, including the upper pole, consisted of a cystic cavity (Fig. 10) which measured 10 cm. in diameter. With the cyst empty, the spleen weighed 380 gm. The cyst fluid was dark red-brown; microscopically it contained a few red blood cells, many granular leukocytes, and a few pigment-containing macrophages. The cystic wall varied in thickness, averaging 0.5 cm. Its inner surface was heavily and coarsely trabeculated by thick, pale bands of tissue. The splenic pulp appeared fairly normal but rather fibrous.

Microscopical sections of the wall of the cyst showed an irregular impocketing of a lining of stratified squamous epithelium (Fig. 11). This covered the whole interior, and there were no areas suggestive of a metaplastic process. The epithelium varied in thickness from several layers to a dozen or more. Some of the surface cells were hydropic, but no distinct keratinization had occurred. Many cells just beneath the surface showed the characteristics of the stratum granulosum. Deeper, the typical prickle cells of the stratum spinosum were found. This epithelium stained yellow with van Gieson's stain. It rested on a dermal layer composed of rather heavy connective tissue which merged imperceptibly into the splenic tissue. Away from the wall of the cyst the splenic tissue was normal in all respects, but became more fibrous as the wall was approached.

Diagnosis. Epidermoid cyst of the spleen.

Summary

A classification of primary splenic tumors based upon histogenesis is presented. Arranged in their order of frequency, as found in the literature, these groups are as follows: (1) vascular—lymphangioma and hemangioma; (2) lymphoid—lymphoma; (3) reticulo-endothelial cells—endothelioma and reticulum cell sarcoma; (4) embryonic inclusions—epithelial cysts, dermoids, and mesothelial inclusion cysts; (5) fibrous tissue—fibrosarcoma; (6) smooth muscle—leiomyosarcoma; (7) nerves—neurosarcoma. No example of a neurosarcoma of the spleen has been found in the literature.

Case reports are presented of: (1) primary reticulo-lymphosarcoma of the spleen in a woman, 90 years old; (2) a primary reticulum cell sarcoma of the spleen; (3) primary splenic endothelioma, two examples; (4) one cavernous hemangioma and one cavernous lymphangioma; (5) epidermoid cyst of the spleen, the eleventh reported in the literature.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

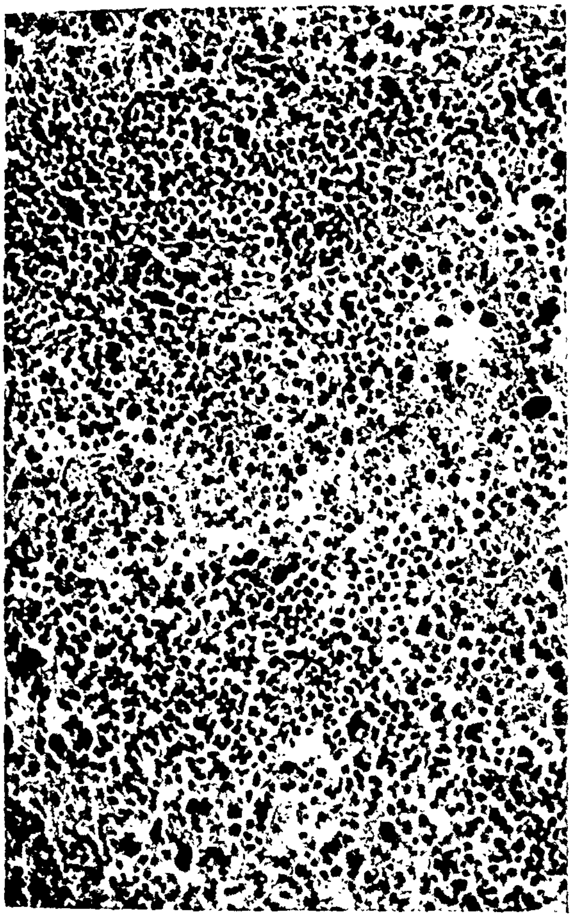
PLATE 201

FIG. 1. (Case CO-42-1313.) Primary splenic reticulo-lymphosarcoma. Large cells of reticulum type can be identified, intermingled with more numerous typical lymphocytes. Mitotic figures are moderately frequent, eosinophilic leukocytes absent. $\times$ 170.

FIG. 2. (Case SP-44-1704.) Primary reticulum cell sarcoma of the spleen. Total splenic weight, 1450 gm. The tumor was almost solitary, with only a few, small, satellite nodules. In many areas it was soft and necrotic and in other areas the tissue was rather dense and fibrous. The cut surface of the tumor at the plane sectioned measured 6 by 9 cm.

FIG. 3. (Case SP-44-1704.) Reticulum cell sarcoma of the spleen. The production of delicate reticulum by tumor cells is seen, as well as their indistinct cytoplasmic outline, pale appearance, and vesicular nuclei. $\times$ 170.

FIG. 4. (Case SP-44-1704.) Primary reticulum cell sarcoma of the spleen showing an area of extensive differentiation into a fusiform cellular type having abundant reticulum and some collagen production (collagen is black). $\times$ 170.

1 

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Bostick

Primary Splenic Neoplasms

PLATE 202

FIG. 5. (Case SP-44-1704.) Primary reticulum cell sarcoma of the spleen showing actively phagocytic tumor cells containing erythrocytes and débris. × 200.

FIG. 6. (Case SS-41-1066.) A field from the primary endothelioma of the spleen, showing a moderate degree of cellular pleomorphism with cells of a rather vesicular, moderate-sized type. Mitotic figures are evident as is the distinct tendency for the neoplastic cells to lie against a delicate fibrous stroma and to line tissue spaces. × 170.

FIG. 7. (Case SS-41-100.) Primary endothelioma of the spleen. The cell type is uniform and cellular outline indistinct. A tendency to line spaces is evident. Mitotic figures are rare. × 170.

FIG. 8. A higher magnification of the field shown in Figure 7. Mitotic figures are not seen and the somewhat syncytial aspect of the cytoplasm is evident. The nuclei have very fine chromatin material and no apparent nucleoli. × 270.

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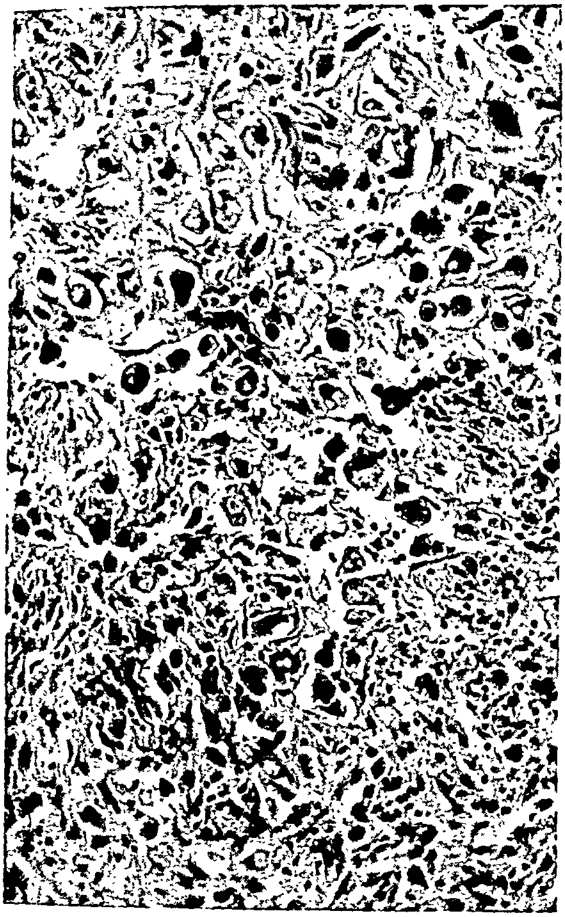

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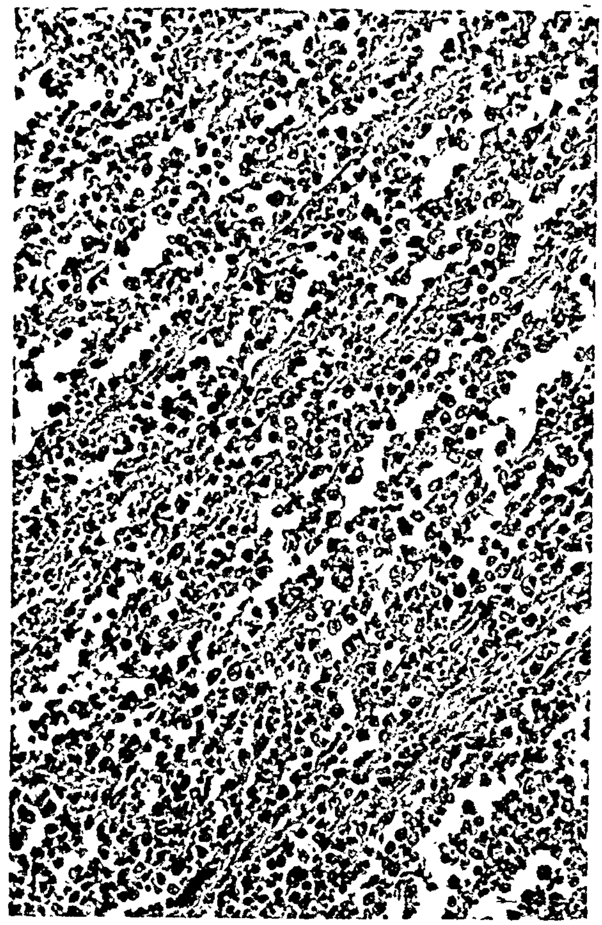

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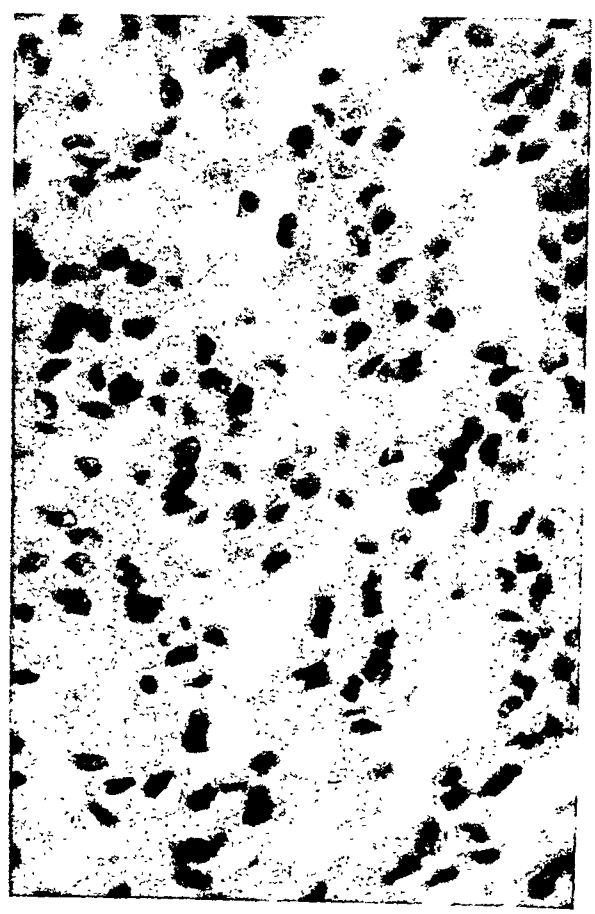

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Primary Splenic Neoplasms

PLATE 203

FIG. 9. (Case SA-35-166.) Cavernous lymphangioma of the spleen. The vascular spaces are filled with light eosinophilic amorphous serum containing a few leukocytes. The lining endothelium is flat and inactive. $\times 170$.

FIG. 10. (Case SP-38-1340.) An epidermoid cyst of the spleen weighing 380 gm. when empty and revealing a single large cavity with coarsely trabeculated wall.

FIG. 11. (Case SP-38-1340.) Photomicrograph of the lining of the epidermoid cyst shown in Figure 10. It consists of polygonal multilayered squamous epithelium. Intercellular bridges are present as well as a stratum granulosum but there is no keratin. $\times$ 170.

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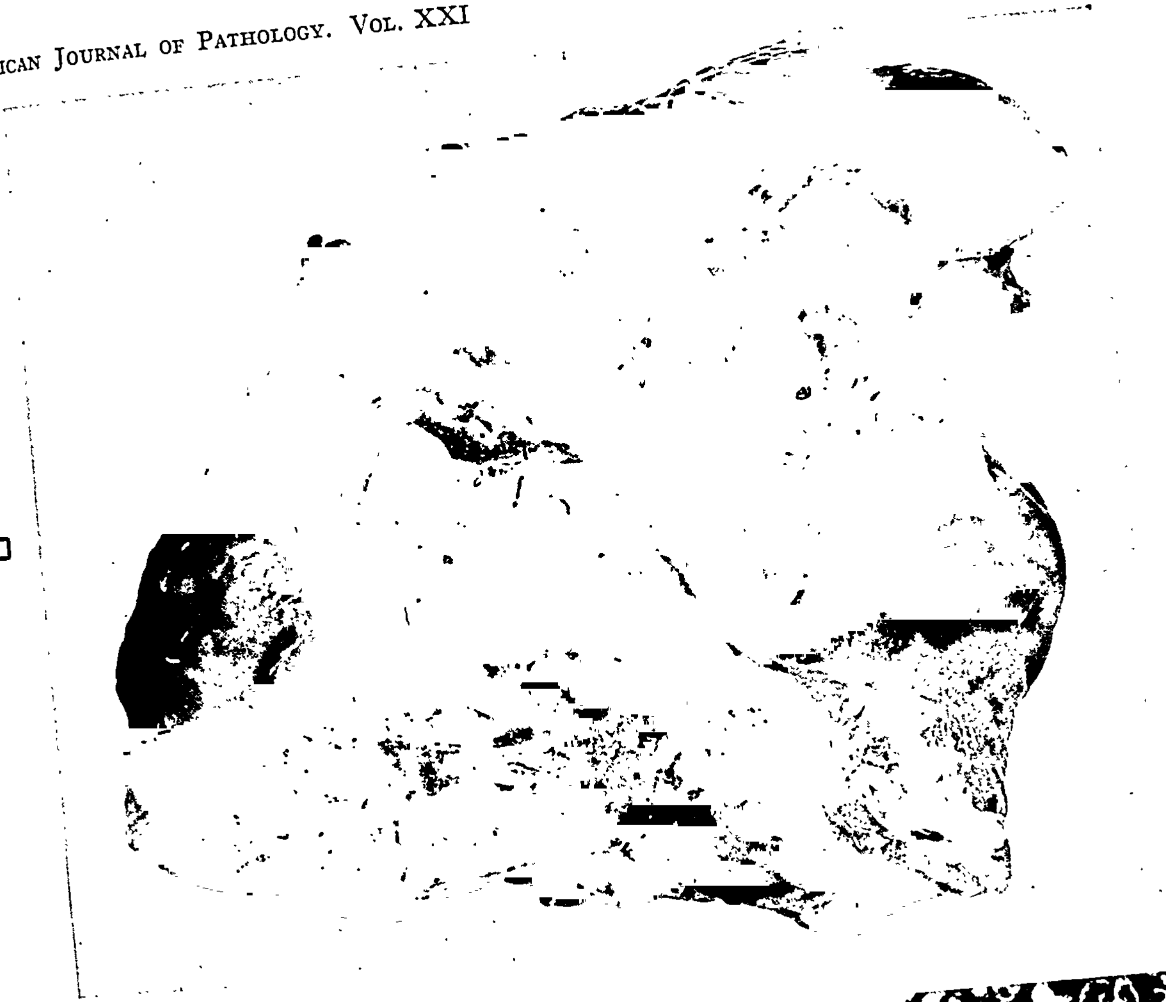

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11

Primary Splenic Neoplasms

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GIANT CYSTIC ARRHENOBLASTOMA OF THE OVARY CONTAINING ENTODERMAL EPITHELIUM AND A CARCINOID *

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Although, since Meyer's [1] fundamental studies, the arrhenoblastomas have been generally accepted as a special group of tumors to be distinguished from other ovarian neoplasms, their origin and nature are still being discussed. Several authors believe that these tumors represent teratomas containing virilizing elements. Kanter and Klawans [2] reported an arrhenoblastoma which contained young, partially developed cartilage, and Krock and Wolferman,[3] analyzing the 70 cases available in the literature, found suggestions of tridermal elements in 24 cases. Other authors, *e.g.*, Geist,[4] considered the teratomatous origin of the arrhenoblastomas as extremely unlikely. Rhoden [5] believed that the difficulty in deciding between a teratomatous and nonteratomatous origin can be avoided if it is kept in mind that in both cases the tumor originates from the sex-cell. However, for this theory no wholly convincing proof has been offered. The case to be reported has three distinguishing features: (1) the enormous size of the tumor; (2) its great pleomorphism; (3) the presence of epithelial formations which could be identified with certainty as entodermal elements.

REPORT OF CASE

A colored girl, native of Aruba, N.W.I., and 20 years old, was sent to the hospital with the following history. Menstruation had started at the age of 16; the flow had always been very scanty and usually consisted only of some "spotting." Fifteen months before, menstruation had stopped completely. She consulted a physician, who on general examination found pronounced hirsutism, atrophy of the breasts, and absence of the normal womanly contours. Gynecological examination revealed a hypertrophic clitoris, about 3½ cm. long, and a pelvic tumor, which could be felt 4 cm. above the symphysis. Operation was refused and several other physicians were consulted. In the meantime the abdomen of the patient enlarged steadily until it surpassed the size of a full-term pregnancy, whereas the general appearance of the patient had become like that of a man. Permission for operation was obtained at last and in view of her general condition laparotomy was performed on the day of her admission into the hospital. An enormous tumor of the right ovary was found. There were no adhesions with the intestines or with the abdominal wall. The uterus and the left ovary showed no pathological changes. The tumor was removed without difficulty (Dr. M. J. Hugenholtz). The postoperative course was uneventful and the patient returned to Aruba after 2 weeks. She was re-examined 3 weeks after the operation, but no change was noted. Eight months after the operation the patient was seen again. She had changed completely and, in the words of her physician, had become a pretty girl. The superfluous hair had fallen out and she had gained weight. Menstruation had occurred 2 months after operation and had been recurring regularly, the flow lasting 5 days.†

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† I am indebted to Drs. J. Oduber and W. de la Fuente for part of this information.

Gross Findings

The specimen consisted of a large, cystic tumor, weighing 12.1 kg. The surface was smooth, the color was bluish red. The tumor contained a slightly turbid, brown-red fluid. The thickness of the cyst wall varied between 4 mm. and 3 cm. The inner lining of the cyst was pale red and slightly irregular; sometimes it resembled hypertrophic endometrium. The thickness of this pale red tissue varied. In several places islands of this tissue were completely enclosed by grayish, edematous connective tissue, which made up the rest of the cyst wall. Several small hemorrhages were noted.

Microscopical Examination

Thin blocks of tissue were fixed in the Bouin-sublimate mixture and embedded in "tissuemat" after treatment with methyl benzoate celloidin. The sections were stained with hematoxylin and azophloxine, hematoxylin and mucicarmine, Petersen's [5a] acid alizarin blue-aniline blue, the azan stain, Masson's tetrachrome, 1 per cent aqueous safranine, phosphotungstic acid hematoxylin in the modification of Masson,[6] and Masson's stain for argentaffine cells.[7]

The wall of the cyst consisted of the neoplastic tissue *sensu strictiori* and of connective tissue; both were present in varying quantities. Sometimes the neoplastic tissue occupied the greater part of the cyst wall but in other places only thin strands of it were found. The tumor consisted of different kinds and types of cells and tissues which very often mingled or merged into each other. In many places the dominating cell type was a relatively small cell with a dark-staining nucleus (Fig. 1). The nuclei of these cells were oval and their longest diameter measured 8 to 12 μ. They contained one or two small nucleoli. There was but little protoplasm, which stained lightly. Since in most places the cells were closely packed, it was very difficult or impossible to distinguish the cell borders. The cells were arranged in smaller or larger groups or alveoli (Fig. 2) which were surrounded by thin septa, carrying thin-walled blood vessels. With the connective tissue stains, very thin, often branching fibers were found inside the cell groups; in many places they surrounded individual cells. It could not be determined with certainty whether the fibers were intraprotoplasmatic. Sometimes the cells merged imperceptibly into small, short, spindle cells. Also groups of cells with paler nuclei were found; intermediate forms between the cell types mentioned were frequent.

In and between the nests of small cells, cells of another, larger type were found. These cells occurred as isolated specimens, in small groups or arranged in cords. These groups and cords were always well de-

limited by connective tissue, resembling a basement membrane. The cells had a distinct cell body with a coarsely vacuolated and reticulated protoplasm; the size of the cells and nuclei varied between wide limits; sometimes giant cells with nuclei measuring 40 μ or more were found (Fig. 3). Density of the chromatin varied. In most nuclei there was a small nucleolus. Intermediate forms between the small and large cells were present. The cords of large cells showed a tendency to form lumina so that more or less regular tubules appeared, lying between the groups of small dark-staining cells. The cells lining the tubules showed, in general, the same reticulated protoplasm, and assumed a prismatic form. In many places terminal bars were found. Sometimes the apical protoplasm of the cells contained very small, closely packed granules. No connective tissue fibers were found inside the basement membrane of these cords and tubules. In other fields the cell cords were broader; the cells had light-staining, band-like nuclei and the same reticular protoplasm (Fig. 4). They were arranged in two to four rows perpendicular to the basement membrane. In the different cell types only a few mitotic figures were found.

At the periphery of, and sometimes also inside, the cellular neoplastic tissue there were larger cells of a different type. They had round to oval nuclei with one, sometimes two, conspicuous nucleoli and a granular protoplasm, which stained dark red with hematoxylin-azo-phloxine and with Masson's tetrachrome, and dark blue with phosphotungstic acid hematoxylin. The protoplasm sometimes contained brown pigment granules, which stained black with silver. In many cells there were small vacuoles. The cells were lying singly or in small groups or rows. When lying in close contact with each other, often a small cleft, completely surrounded by protoplasm, was observed, producing a certain resemblance to liver cells and bile canaliculi (Fig. 5). These cells were also found in combination with another type of tissue, which will be described later.

Apart from the tubules which were found between the cells of the small type, other tubules were found lying in groups in the connective tissue of the cyst wall. Their lining epithelium was columnar and very regular; the apical protoplasm of the epithelial cells was finely granular, the basal protoplasm was more clear; the lumina often contained acidophilic granules or more homogeneous material (Fig. 6).

Cells of another type remain to be described, which, though present in small numbers in many places, completely dominated in others. They were relatively large cells with oval, more or less curved, bean-shaped or hook-shaped nuclei of medium density. The cells occurred as isolated elements or were arranged in small groups or cords. When

only two cells were lying together, the concave side of the nuclei faced each other. The isolated cells or the cell groups and cords were always surrounded by a fairly thick connective tissue membrane. In several places a large part of the cyst wall was almost exclusively occupied by closely packed, straight or curved cords of these cells, the cords being separated by thin septa of connective tissue (Figs. 7 and 8). The connective tissue which formed part of the cyst wall was edematous and contained fibrocytes, unstriated muscle cells, which were often arranged in bundles, and loose bundles of collagen fibers. In this connective tissue there were peculiar cell complexes which deserve a more detailed description. The complexes consisted of relatively small stellate cells with round to oval nuclei containing a small nucleolus. The cells were connected with one another by their processes. Very thin fibers, staining with aniline blue, were found in the protoplasm of these cells. The spaces between the cells appeared to be empty. Sometimes the cells lined cystic spaces, which contained little granular material. The whole structure resembled embryonal mesenchyme or the anlage of a lymph node. The stellate cells showed a tendency to be transformed into short spindle cells, which were lying close together, or into the larger cells, which resembled liver cells or interstitial cells (Figs. 9 and 10). Other cells remained smaller; their protoplasm was partly vacuolated, partly granular; a very few cells contained pigment reducing silver; their nuclei, however, resembled those of the larger cells; they occurred also in small groups and cords in the connective tissue. These cells resembled the interstitial cells which have been described in the hilum of the ovary (Fig. 11).

In one place a cystic space with a diameter of 1.5 mm. and containing a granular precipitate was found; it was partially lined by tissue composed of closely packed spindle cells (Fig. 12), small rounded cells, and interstitial cells; where the lining was thin it showed a superficial resemblance to an ovarian follicle.

In several blocks there were tubules and cysts which, though almost everywhere surrounded by, and in close contact with, the neoplastic tissue described above, were lined by an epithelium of a completely different type (Fig. 13). This epithelium varied from cuboidal to columnar and was in most places very regular; the nuclei were round, oval, or elongated and often contained conspicuous nucleoli. The free surface of many cells was covered by a striated border, under which there was a zone of homogenous protoplasm (Fig. 14). There were typical terminal bars. Here and there mitotic figures were found. Scattered between the epithelial cells were typical goblet cells with their thecae and stomata; they gave a positive reaction with mucicarmine.

Apart from the epithelial cells with and without a striated border and the goblet cells, a fourth epithelial cell type was found. These cells were also scattered between the other epithelial cells. The body of the cell sometimes made a bulge into the basal membrane; the cell body and nucleus were smaller than those of the other epithelial cells and the nucleoli were mostly very small or absent. The cells were more or less flask-shaped or triangular, the base of the cell resting on the basal membrane, the tip sometimes, but not always, reaching the surface. Sometimes the cells were more cylindrical.

In the basal protoplasm of these cells and only rarely higher than the nucleus, small yellowish granules were found. These granules stained deep orange-yellow with phosphotungstic acid hematoxylin and bright reddish yellow with safranine. With Masson's silver stain they stained brown-black (Figs. 15 and 16). Most of the tubules and cysts were completely surrounded by neoplastic tissue in which cells of the smaller type dominated. Others were lying in loose connective tissue. In this connective tissue there were larger and smaller groups of cells unlike any found in other parts of the tumor. The cells were smaller and often rounded or cuboidal, especially the cells lying in the outermost layer of the group. In the larger cell groups there were small lumina around which the cells were arranged in pseudorosettes (Fig. 17). Many of the cells, especially those bordering on the connective tissue, contained fine argentaffine granules (Fig. 18); with phosphotungstic acid hematoxylin they stained orange-yellow, as did the argentaffine cells found in the epithelium of the cysts and tubules.

Discussion

The diagnosis in this case cannot be considered difficult for almost all types of cells and tissues hitherto described in arrhenoblastomas were present. There were not only very typical tubules and interstitial cells, but also "sex cord-like" formations, atypical tubules and "sarcoma-like" areas (Fig. 12). The tubules depicted in Figure 4 resembled the tubules of the embryonal testicle, with the important difference that the conspicuous primitive sex-cells were absent. Formations like those in Figure 8 are depicted by Ewing[8] as testicular adenoma of the ovary. The interstitial cells were also quite typical; they contained silver-reducing pigment and resembled the interstitial cells of the embryonal testicle. Their origin from mesenchyme-like cells could be easily observed. Little importance can be attached to the fact that they did not contain crystalloids; in cases of tumor-like hyperplasia of the interstitial cells, crystalloids are often absent,[9, 10] and their occurrence in the interstitial cells of the normal testicle is not constant. The resemblance to liver cells has already been emphasized by Snell-

man[9] and Kaufmann.[11] The smaller vacuolated interstitial cells resembled the cells described by Berger[12] and Neumann[13] in close contact with nerves in the hilum of the ovary. The tubules with the regular columnar or cuboidal epithelium (Fig. 6) are identical with those found by Kleine[14] in several cases of arrhenoblastoma; they do not resemble structures found in the normal ovary or testicle.

The areas of small cells with darker or paler nuclei could be erroneously diagnosed as belonging to the diffuse variety of granulosa cell tumor. The presence of the numerous connective tissue fibers between the cells speaks against this diagnosis and Schiller[15] has pointed out that granulosa cell tumors and arrhenoblastomas cannot be differentiated from each other in the first immature phases. It must therefore be assumed that in my case both very typical and very immature neoplastic tissues were present; this is further proved by the finding of the mesenchyme-like areas. There were also many intermediate forms between the different cell types.

The tubules and small cysts, lined by the cuboidal or columnar epithelial cells with the striated border and by goblet cells, belong to an absolutely different type of tissue. The epithelium could not be distinguished from that of the intestine or other derivatives of the entoderm. That they were really of entodermal origin was proved by the presence of argentaffine cells. These cells occur only in the stomach, intestine, pancreatic duct, and gallbladder.[16, 17] As follows from the description and the photomicrographs, the cells in my case possessed all the characteristics of the argentaffine cells of the intestine, especially in regard to cellular form and to location and nature of the granules, which gave the typical staining reaction with Masson's silver method and with safranine. They were also very well brought out by phosphotungstic acid hematoxylin. The smaller and larger cell groups with the pseudorosettes and the argentaffine cells can only be diagnosed as a carcinoid; the location of the argentaffine cells in the different cell groups is typical for these tumors (see Masson's[7] Plate 6, Figs. 3 and 4). Whereas, for example, cartilage and unstriated muscle fibers can, at least in theory, develop everywhere in the mesenchyme and their presence be thus easily explained, this is not the case with entodermal epithelium and carcinoids. Black[18] and Mechler and Black,[19] who described a case of gynandroblastoma in which tubules with a more atypical epithelium showing a striated border and goblet cells were found, and which they did *not* compare with intestinal epithelium, adhered to the theory that the gynandroblastomas are teratomatous. Carcinoids have been found three times in ovarian tumors; these tumors were all dermoids—teratomatous tumors.[20] It is difficult to find

an explanation for the presence of the entodermal epithelium and the carcinoid in my case of arrhenoblastoma other than that of its teratomatous nature. When it is remembered that the pseudomucinous cystadenomas and the strumata ovarii are considered to be teratomas in which the pseudomucinous epithelium [21] or the thyroid tissue has dominated or blotted out the other teratomatous elements, there is nothing against assuming that in my case male-directed, hormone-producing elements have done the same, especially since Peyron [22] has proved that gonads may occur in a teratoma. Cases like that of Van Bouwdijk Bastiaanse,[23] in which an arrhenoblastoma was found in the wall of a pseudomucinous cyst, could be explained in the same manner. The origin of the teratoma itself remains, of course, open to discussion.

Summary

In a very large, cystic arrhenoblastoma of the ovary entodermal elements, consisting of tubules and small cysts lined by epithelial cells with a striated border, goblet cells, and argentaffine cells, and a carcinoid were found. The entodermal elements were nearly everywhere surrounded by, and in close contact with, the cells belonging to the arrhenoblastoma. The case offers strong evidence for the theory that the arrhenoblastomas are of teratomatous origin.

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DESCRIPTION OF PLATES

The eighteen photomicrographic illustrations which follow were all prepared from the cystic arrhenoblastoma of the ovary which forms the subject of this report.

Plate 204

Fig. 1. Cells of the small type which were the dominant element in many areas. × 715.

Fig. 2. The small cells in an alveolar arrangement. × 300.

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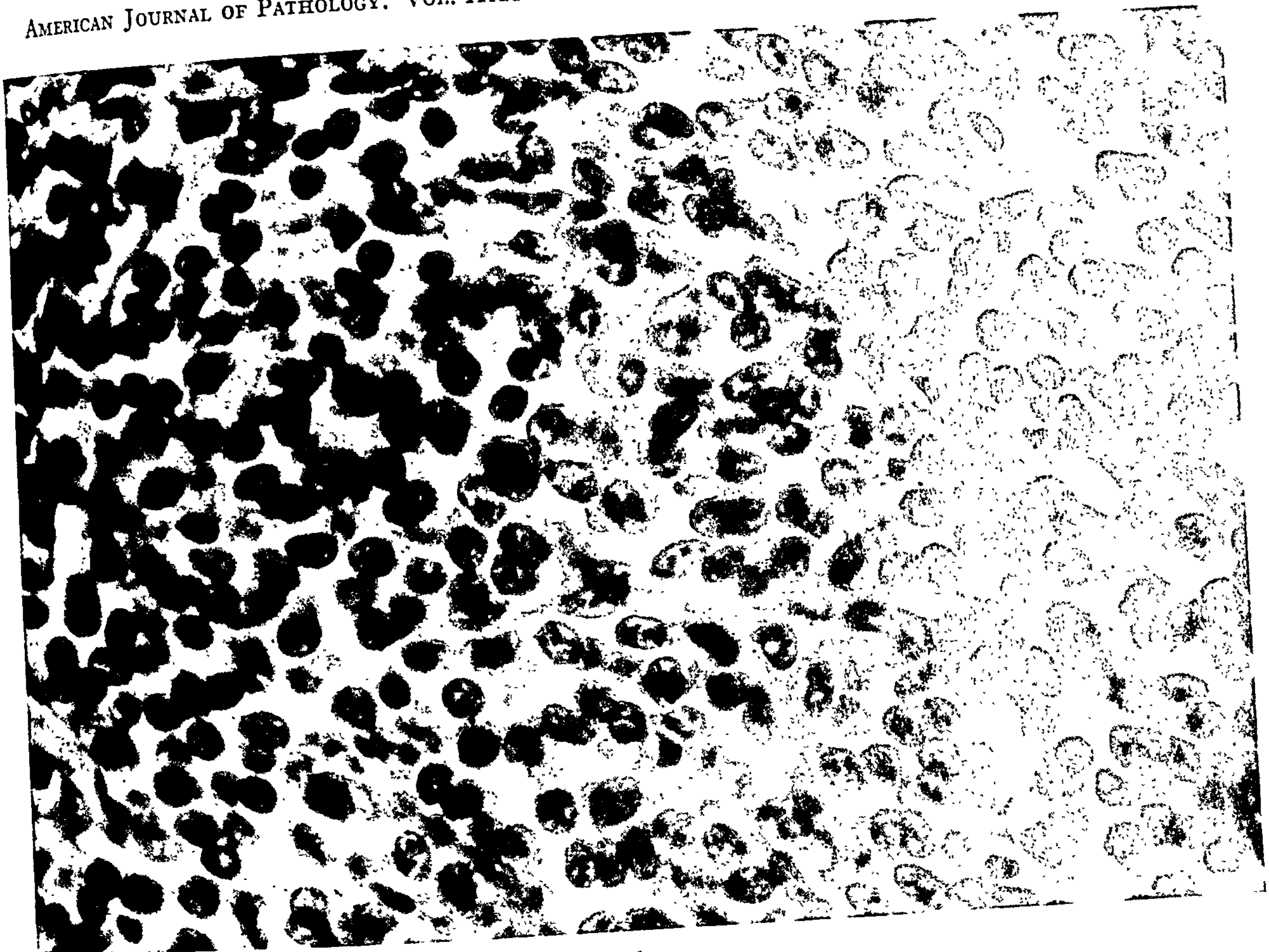

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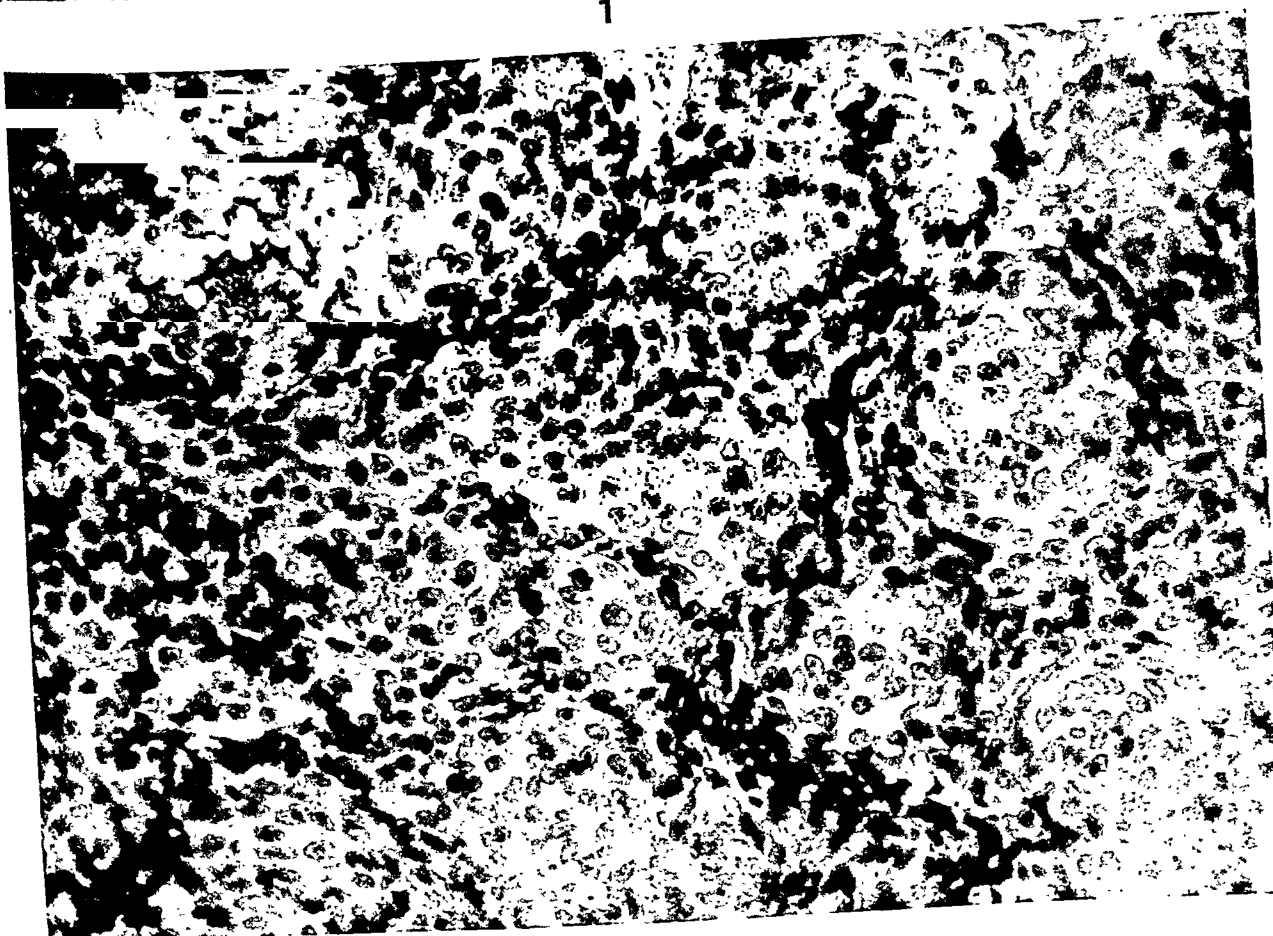

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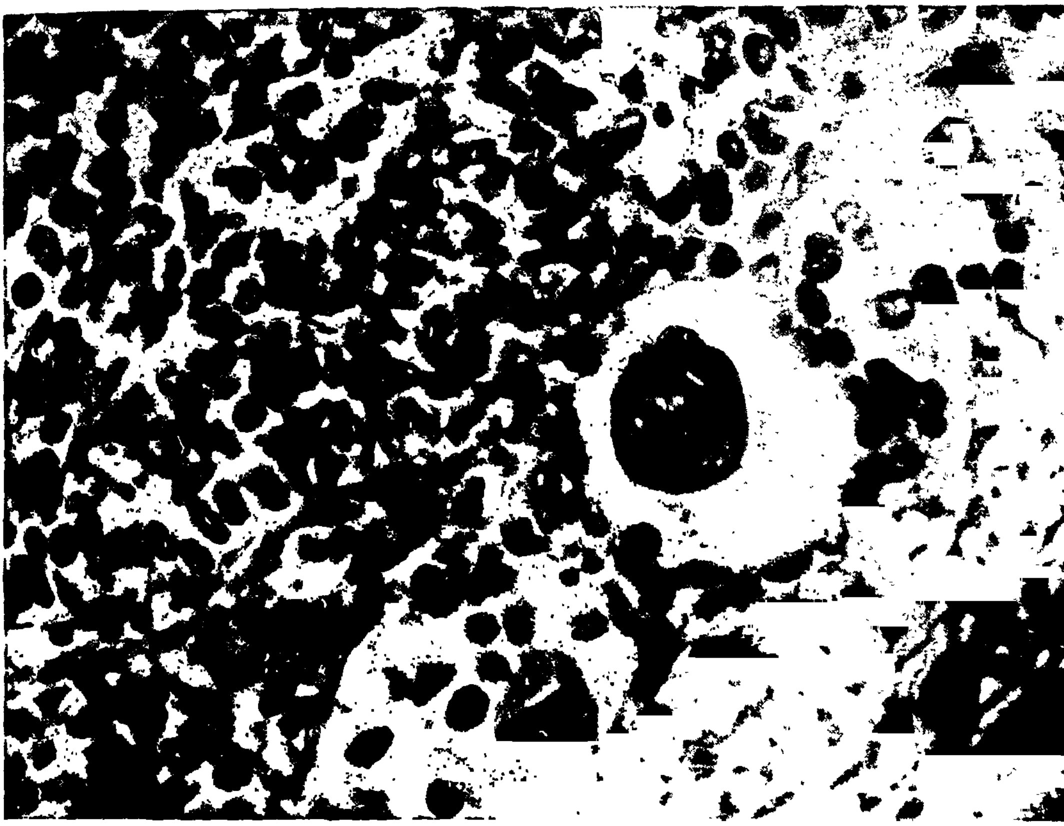
Hartz

Arrhenoblastoma with Entodermal Epithelium

PLATE 205

FIG. 3. The giant cell form of the larger cellular type. × 715.

FIG. 4. Tubules resembling embryonal testis. × 715.



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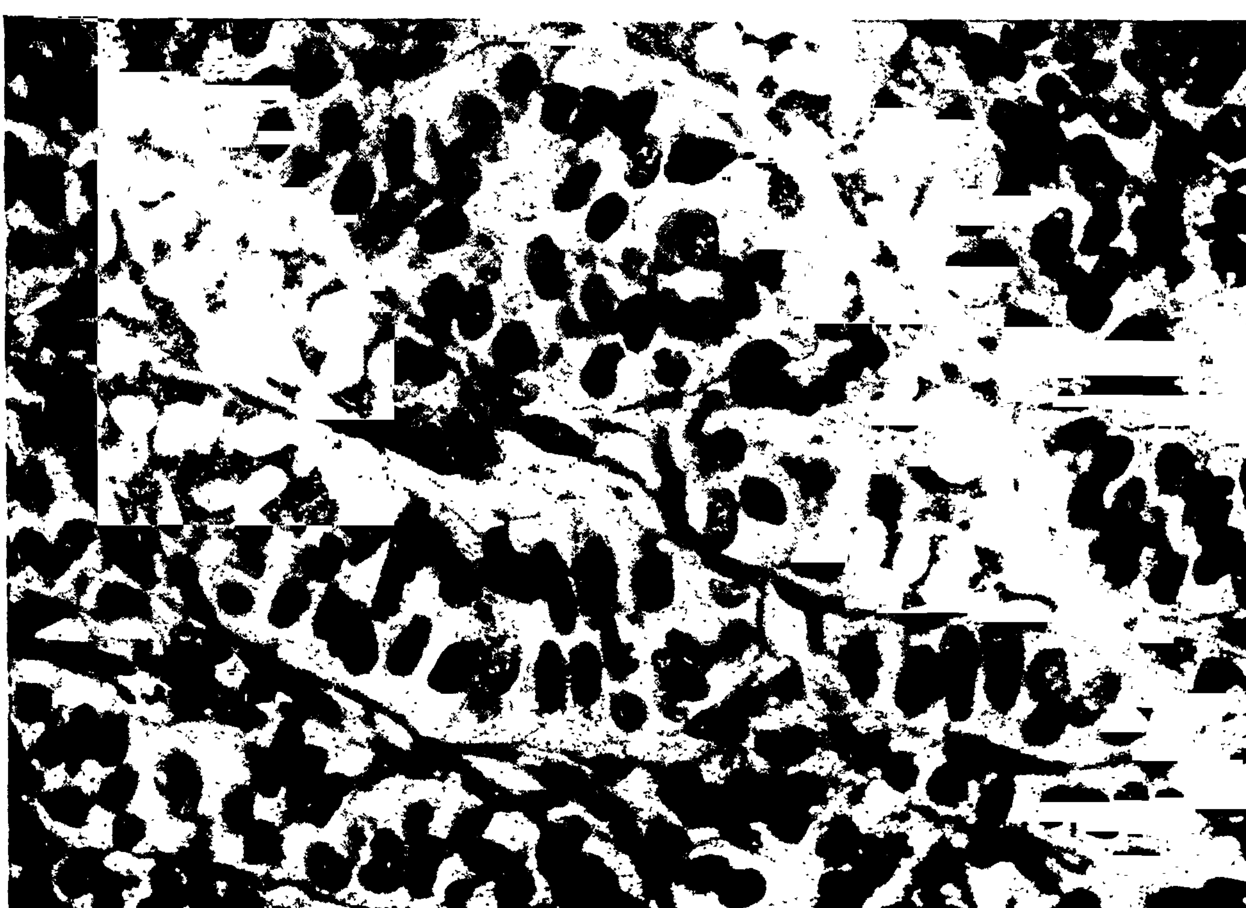
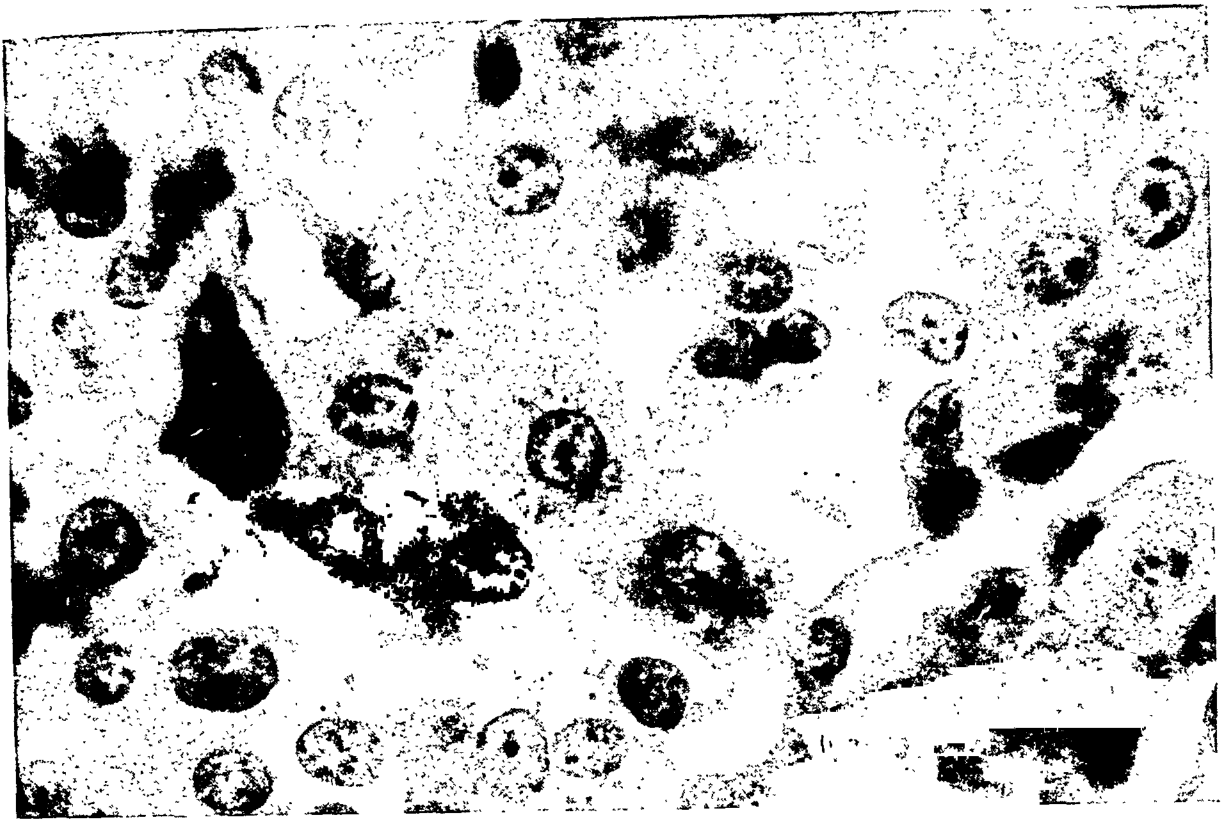


Hartz 4 Arrhenoblastoma with Entodermal Epithelium

PLATE 206

FIG. 5. Interstitial cells containing isolated pigment granules. × 1275.

FIG. 6. Atypical tubules. × 610.



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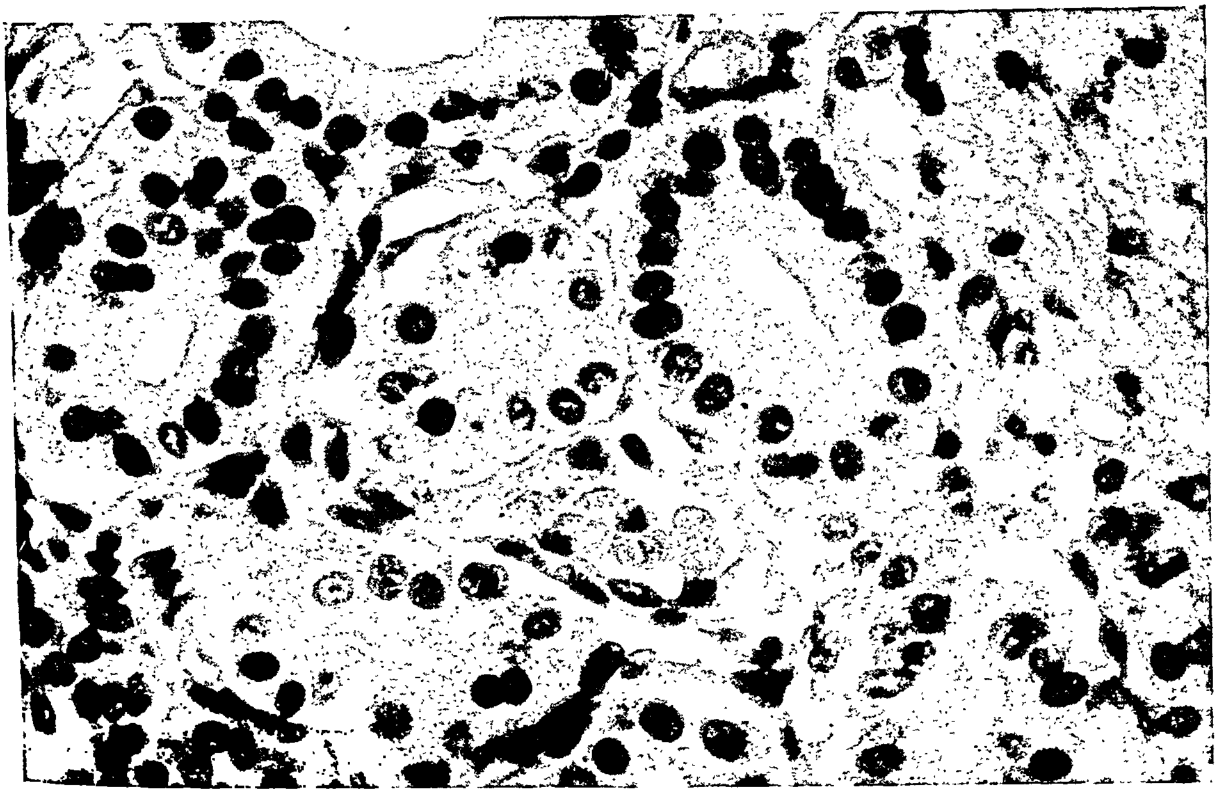

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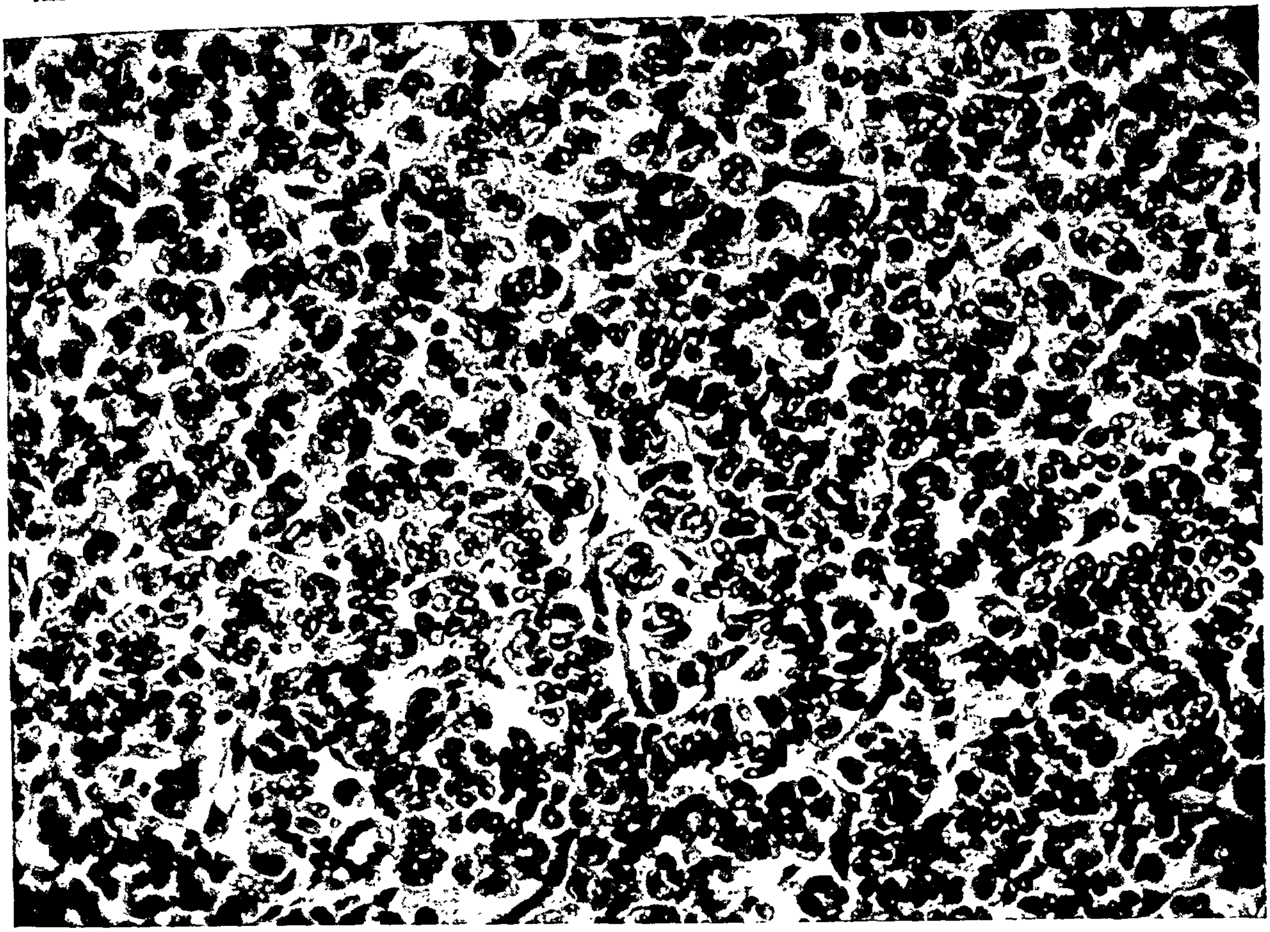
Arrhenoblastoma with Entodermal Epithelium

PLATE 207

FIG. 7. "Sex cord-like" formations. × 300.

FIG. 8. "Sex cord-like" formations. Acid alizarin blue-aniline blue stain. × 715.



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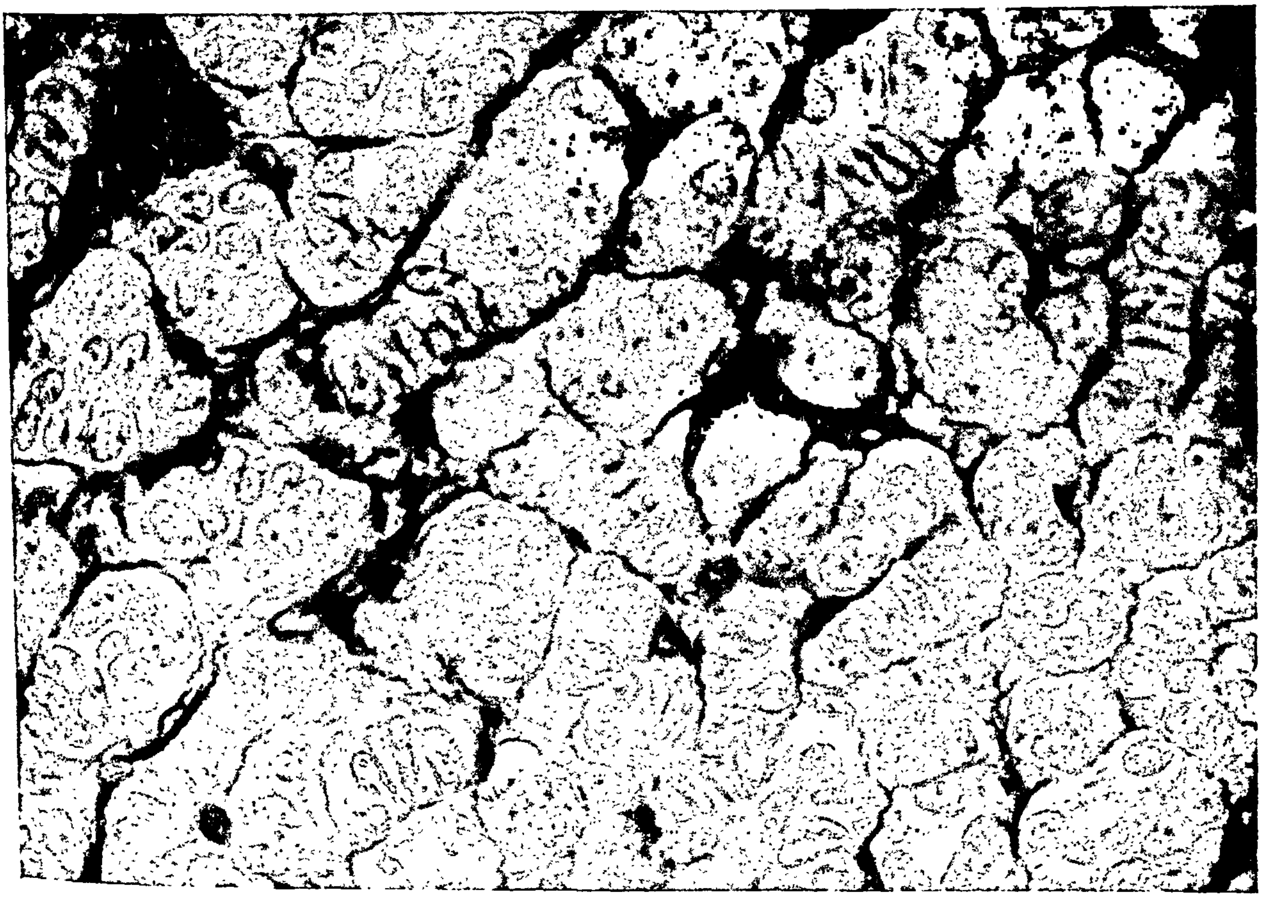

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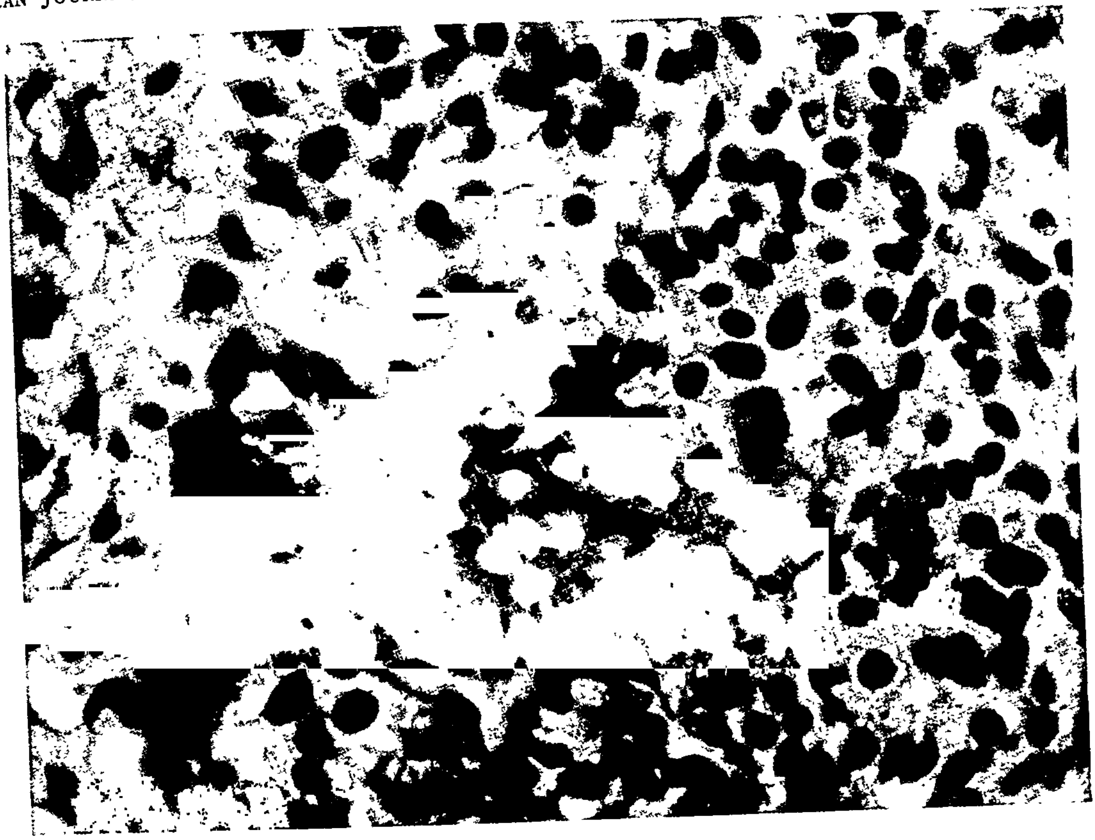
Arrhenoblastoma with Entodermal Epithelium

PLATE 208

FIG. 9. Interstitial cells, resembling liver cells, in a mesenchyme-like area. × 400.

FIG. 10. Interstitial cells differentiating from mesenchyme-like cells. × 1500.



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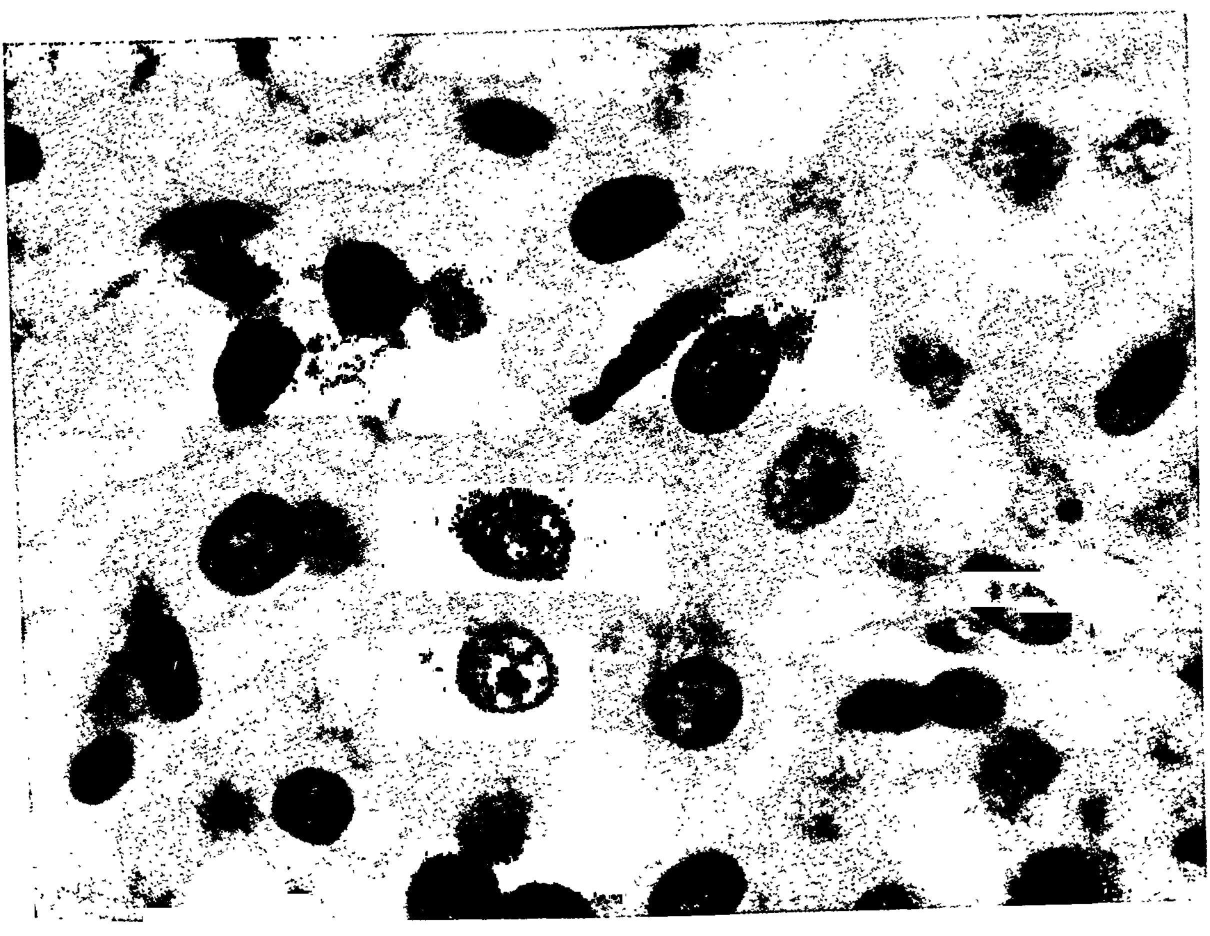

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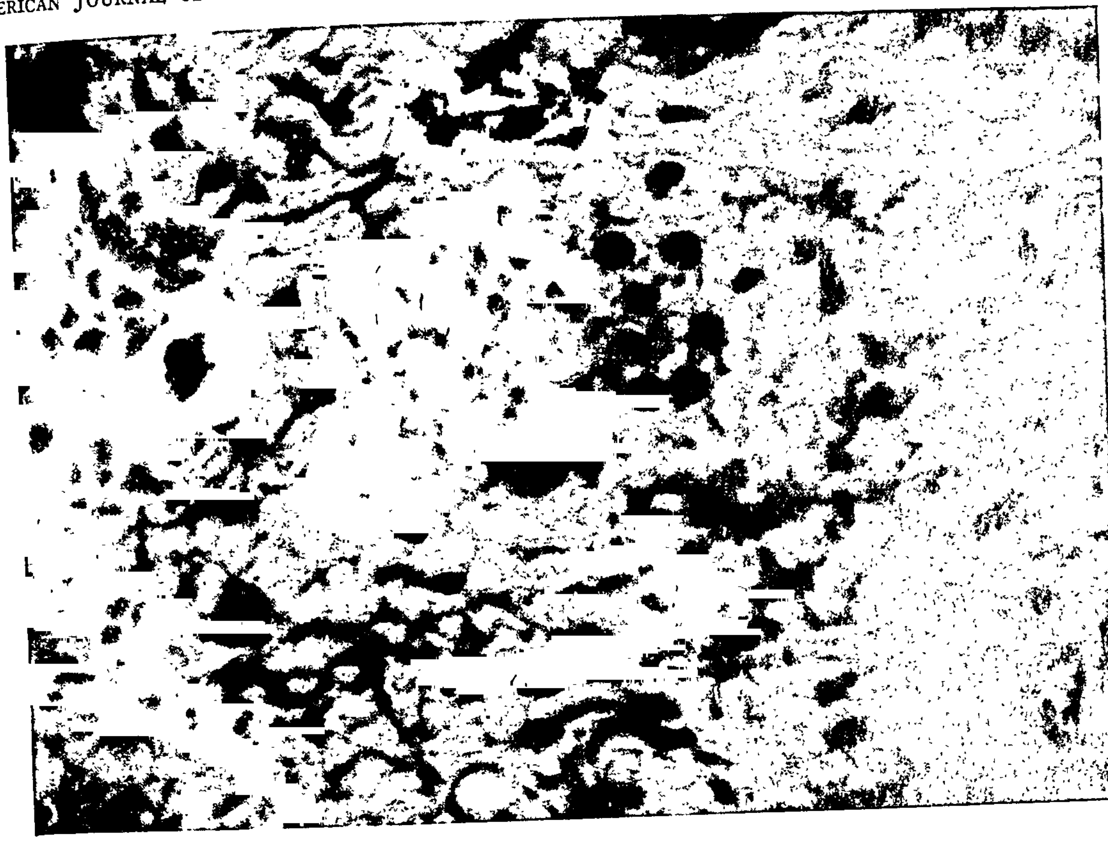
Hartz Arrhenoblastoma with Entodermal Epithelium

PLATE 209

FIG. 11. A group of small, vacuolated interstitial cells. × 400.

FIG. 12. A sarcoma-like area, with short spindle cells and one interstitial cell. × 715.



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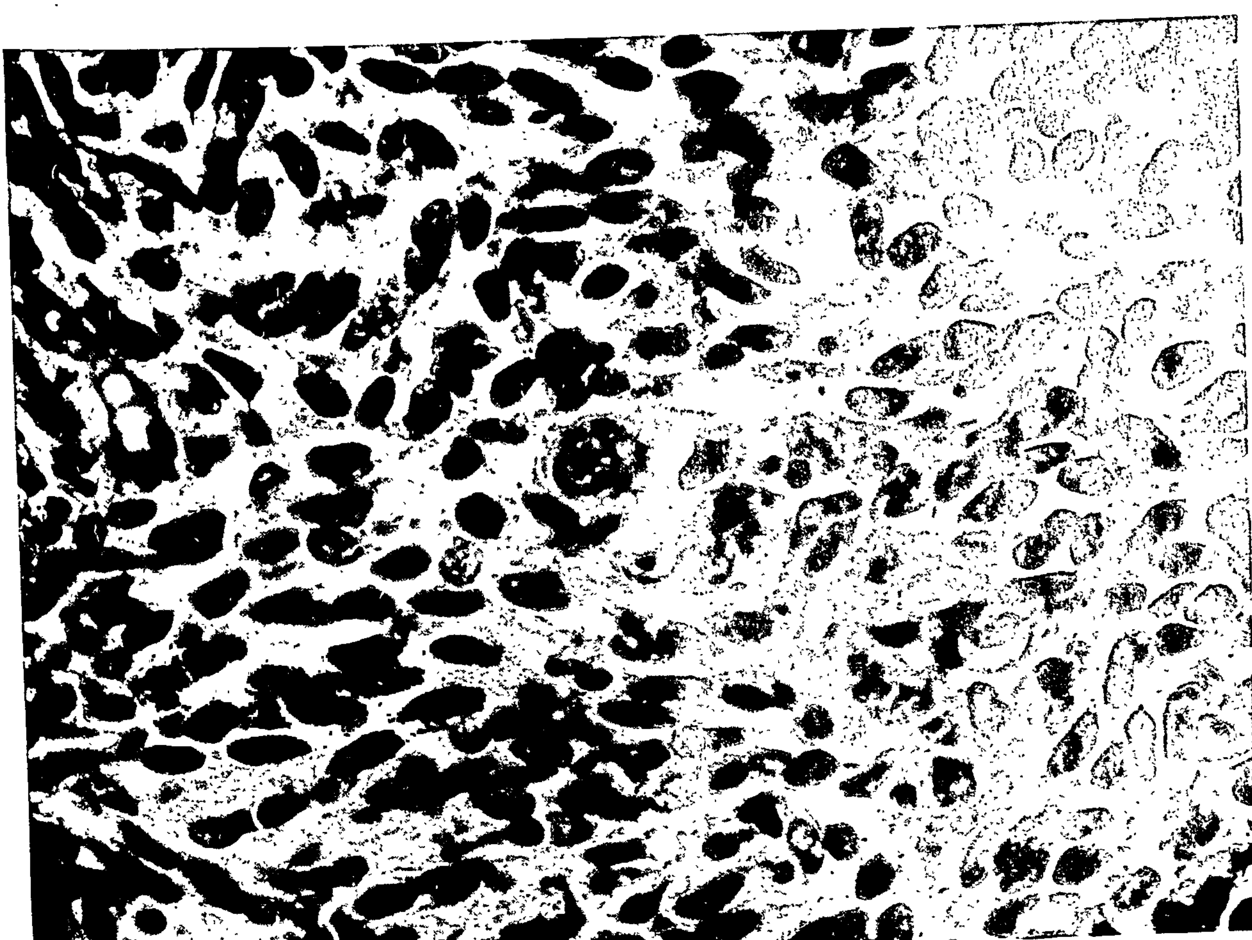

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Arrhenoblastoma with Entodermal Epithelium

PLATE 210

FIG. 13. Wall of a cyst lined by entodermal epithelium and surrounded by neoplastic tissue of the small-celled type. $\times$ 260.

FIG. 14. High prismatic epithelium with a striated border. $\times$ 625.

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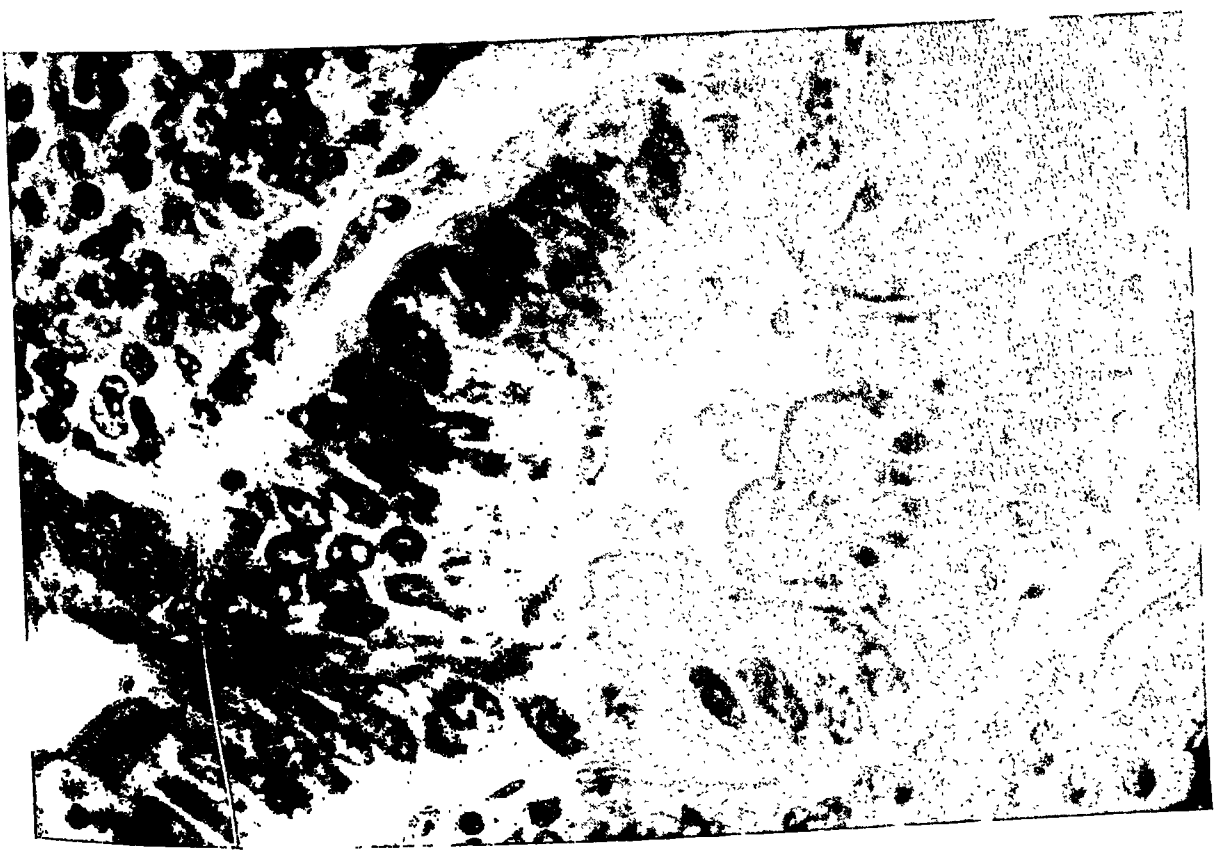

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Arrhenoblastoma with Entodermal Epithelium

PLATE 211

FIG. 15. Entodermal epithelium containing argentaffine cells and surrounded by neoplastic tissue of the small-celled type. Masson's silver stain. × 500.

FIG. 16. Entodermal epithelium with typical argentaffine cells. Masson's silver stain. × 1250.

15

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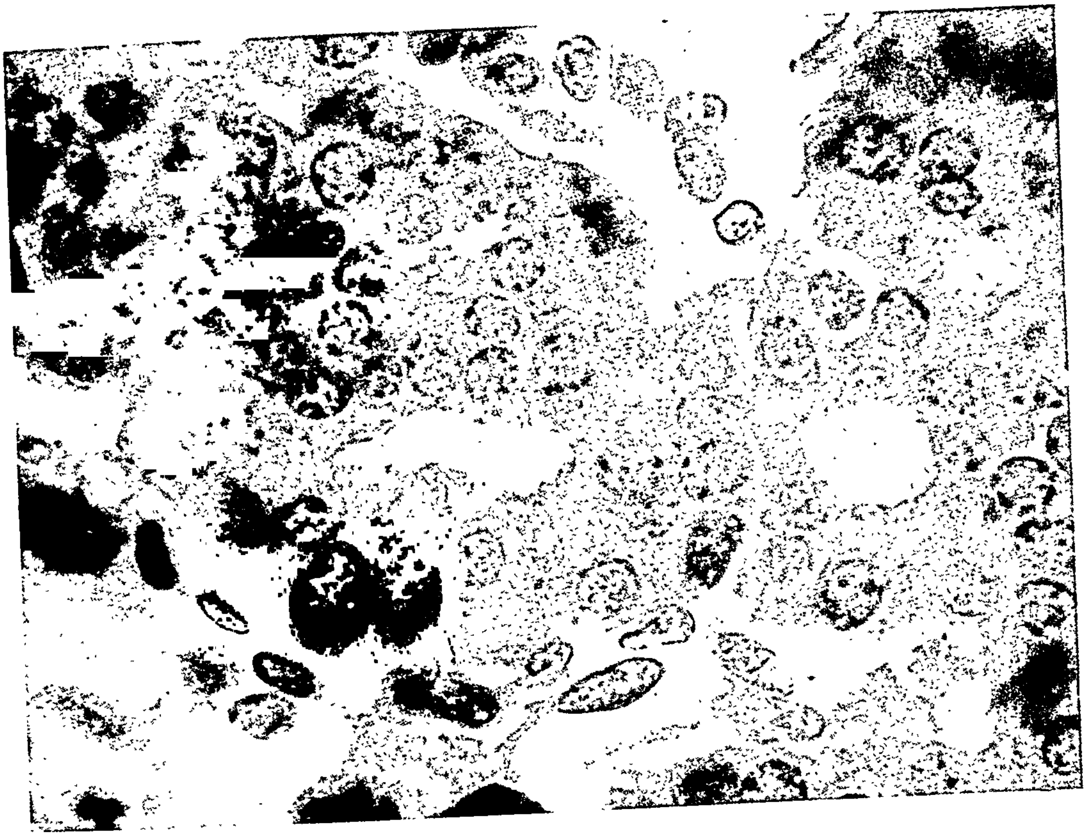
Arrhenoblastoma with Entodermal Epithelium

Plate 212

Fig. 17. The carcinoid, showing pseudorosettes. Phosphotungstic acid-hematoxylin stain. $\times$ 900.

Fig. 18. The carcinoid, showing argentaffine cells. Masson's silver stain. $\times$ 900.



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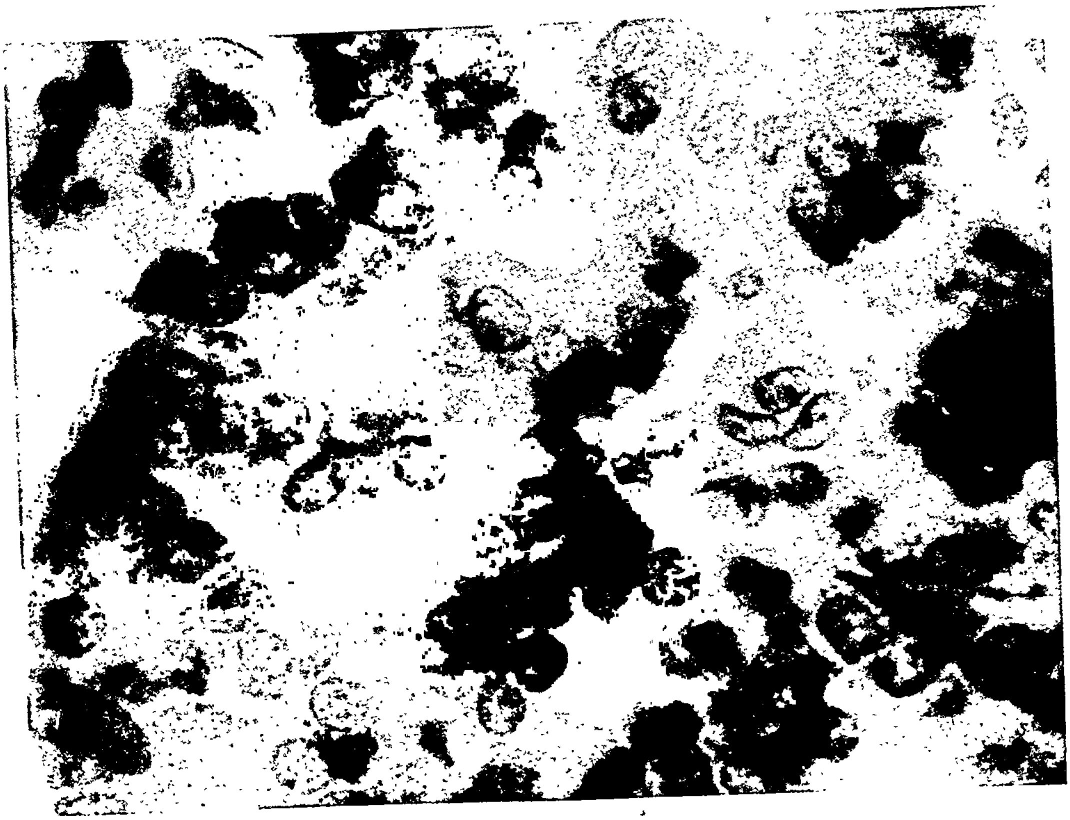

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Hartz

Arrhenoblastoma with Entodermal Epithelium

TUBERCULOSIS OF THE MYOCARDIUM CAUSING COMPLETE HEART BLOCK *

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The rarity of tuberculosis of the myocardium is well established (Norris,[1] Raviart,[2] Horn and Saphir [3]). Gouley, Bellet, and McMillan [4] found approximately 200 cases in the literature. But tuberculosis of the myocardium involving the interventricular septum and causing heart block is so rare that the following case is of special interest.

Report of Case

Y. B., a well nourished woman of 35 years, was admitted to the Second Physician's ward of the King George Hospital on January 8, 1941. She complained of a peculiar sensation in the precordium, giddiness, and fainting fits of 7 months' duration. There was a previous history of intermittent fever for 2 months. During the fainting attacks there was no incontinence of sphincters nor a history of biting the tongue. Convulsive movements were also absent. On admission, the pulse was slow, bigeminal in type, with good volume and tension, the rate ranging from 30 to 40 per minute. There was no difference in the volume or rhythm on change of posture or exercise. Venous pulsation was noticeable at the root of the neck. The blood pressure varied from 170/110 to 154/90 mm. of Hg. The apex beat was not seen, but was felt in the fifth interspace in the midclavicular line and on percussion the left border was in the same position, other borders being normal. The heart sounds were slow. There was one ventricular extrasystole after every normal beat (coupled beats). A soft systolic murmur, heard at the mitral area, was conducted to the axilla. Other systems were largely normal. The urine showed more than a trace of albumin. The radiogram showed a prominent pulmonary conus with enlargement of the first part of the aorta. The patient died during an attack on April 8, 1941.

Gross Examination

Autopsy (Dr. C. S. Raju) showed a normal pericardium and a heart with increased fat under the epicardium. The weight of the heart was about 320 gm. There was a slight dilatation of the right auricle and ventricle and the myocardium appeared pale and had undergone fatty degeneration. The tricuspid ring was somewhat dilated. The tricuspid and pulmonary valves were normal. The left auricle and the mitral opening were of normal size. The aortic cusp of the mitral valve was slightly thickened and opaque and showed irregular calcified areas. Similar calcified opacities were found in the aortic cusps below their free margins. The coronary arteries showed moderate atheroma. A section of the interventricular septum revealed two whitish nodules in the muscle. One, vertically oval, measured 8 by 4 mm. and was near

* Received for publication, November 24, 1944. Because of difficulties in transportation, proof was not read by the authors.

the junction of the septum with the pars membranacea. The other was about 13 mm. below, in the thick muscular part of the septum. It was a horizontally oval mass measuring 4 by 2 mm. In serial sections these two nodules appeared irregularly continuous in the anterior part of the septum. The nodules were whitish yellow, firm, not encapsulated, but faded off into the surrounding pale myocardium. The tissue between the nodules was pale brown and rather friable, with small paler areas apparently in continuity with nodules. Further examination of the extent of the nodules could not be made without impairing the specimen.

The lungs showed some venous congestion, especially at the bases and posterior borders. There were pleural adhesions over the left lung but no nodules could be made out on section. The hilar lymph nodes showed only pigment deposit. The liver was normal in size and showed a smooth capsule, subcapsular mottling, and a faintly nutmeg appearance on section. The spleen was also normal in size and showed two old infarcts. The kidneys were slightly congested. Two small submucous leiomyomata were present in the uterus. The stomach and intestine showed nothing abnormal. There was slight congestion of the brain. Death was attributed to heart block followed by right heart failure. The nodule in the interventricular septum was regarded either as a gumma or a tuberculous nodule.

Microscopic Examination

The muscle fibers in the interventricular septum showed some increase in lipofuscin. A thin layer of hyalinized muscle fibers was found under the endocardium, but deeper down the muscle tissue was replaced by an irregular ramifying network of glistening collagen fibrils interspersed with lymphocytes and mononuclear cells with necrotic muscle fibers at the edges. There were numerous multinucleated giant cells, some of the typical Langhans' type and some of a more irregular foreign body type. Masson's stain showed that the fibers were of connective tissue type, staining with aniline blue. The giant cells in places were in clusters of two or three surrounded by ramifying fibers with a few lymphoid cells in between. In other areas the giant cells were arranged in systems of typical tuberculoid follicles. The possibility of a parasitic granuloma was considered, but no foreign material could be detected inside or outside the giant cells in serial sections. Small focal areas of lymphoid and mononuclear infiltration were found in the muscle bundles away from the granuloma. No endarteritis could be made out in the branches of the coronary vessels. Tubercles with

typical epithelioid cell-clusters and beginning caseation were more common in the periphery of the granuloma, while a more diffuse fibroblastic network was found in the center of the mass. Doubly refractile bodies could not be made out inside or outside the giant cells nor were fungal elements demonstrable. Staining by Jahnel's method showed no spirochetes. Examinations were made for acid-fast bacilli by various modifications of the Ziehl-Neelsen stain. They were found in scanty numbers lying free in the reticular mesh. Acid-fast bacilli were also independently demonstrated by Professor N. G. Pandalai of the Department of Bacteriology, so that the tuberculous nature of the granuloma was beyond question. Subsequently a careful histologic study of sections of various parts of the lungs showed a small tuberculous focus in the left lung lying under the pleura close to the apex.

Comment

Large caseous or conglomerate tuberculous lesions when found in the heart are generally in the auricular wall (Raviart,[2] Anders [5]) and are the result of extension from the hilar glands with pericardial involvement. Isolated myocardial foci deep in the muscle without pericardial involvement are generally regarded as due to hematogenous spread unless the endocardium is primarily involved. Even in valvular tuberculosis, involvement of the myocardium is not common. Baker [6] found only one instance in six of his reported cases. While continental authors regard subendocardial nodules as not very uncommon in general miliary tuberculosis, deep myocardial tubercles are rarely found. This rarity of deep myocardial tuberculosis may be due to the difficulty of development of a focus in an active contractile tissue.

A feature of interest in this case is the probable hematogenous origin since no pericardial or endocardial focus could be demonstrated after careful examination. The only other focus in the body which could be regarded as histologically active was a subpleural nodule. There was nothing to suggest that this was secondary to rupture of the myocardial lesion. The presence of this subpleural focus rules out the possibility of a primary myocardial tuberculosis such as was reported by Berger and Miller,[7] by Gunewardene and Gunewardene,[8] and others. The lack of involvement of the mediastinal glands also suggests an accidental myocardial localization from the blood stream. Wilbur [9] has reported four cases of myocardial tuberculosis, in two of which the infection was apparently hematogenous. The tubercles appeared in small scattered foci and in one case healing had given rise to interstitial myocarditis. The location of the nodules in the present case in the

interventricular septum, giving rise to complete heart block, is a feature of great interest, while the absence of tubercles in the rest of the myocardium is also a noteworthy feature.

The patient was admitted under the care of one of us (C.K.P.R.) but subsequently, for a short while, came under the care of Dr. R. Viswanathan to whom our thanks are due.

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DESCRIPTION OF PLATE

PLATE 213

FIG. 1. Two tuberculous nodules in the interventricular septum. The right ventricle has been removed.

FIG. 2. Tuberculoid follicles at the periphery of the granuloma in the heart muscle. Hematoxylin and eosin stain. × 80.

FIG. 3. Subpleural nodule with tubercles. Hematoxylin and eosin stain. × 80.

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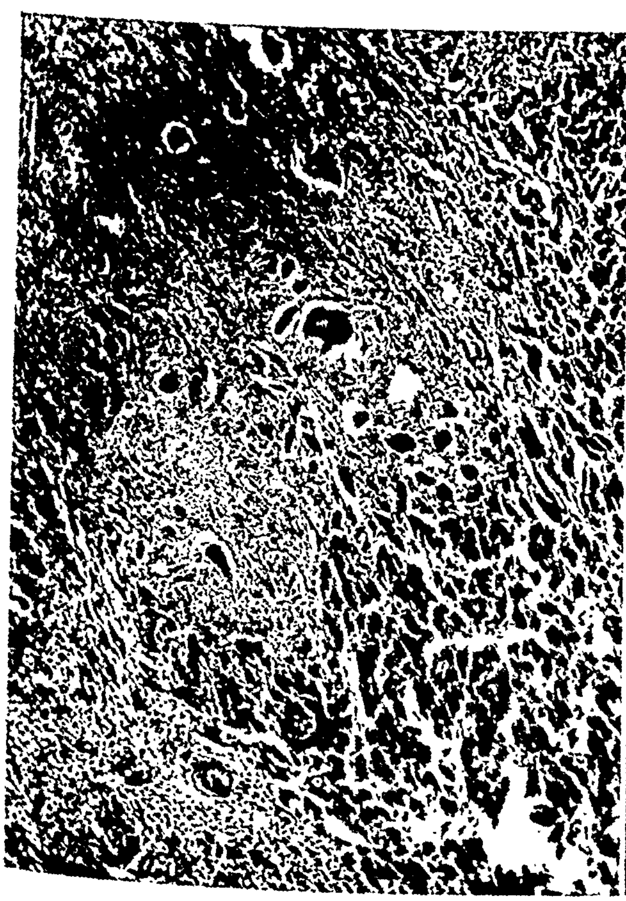

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